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# Differential inhibition of noradrenaline release mediated by inhibitory $A_1$-adenosine receptors in the mesenteric vein and artery from normotensive and hypertensive rats

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ABSTRACT

Mesenteric arteries and veins are densely innervated by sympathetic nerves and are crucial in the regulation of peripheral resistance and capacitance, respectively, thus, in the control of blood pressure. Presynaptic adenosine receptors are involved in vascular tonus regulation, by modulating noradrenaline release from vascular postganglionic sympathetic nerve endings. Some studies also suggest that adenosine receptors (AR) may have a role in hypertension. We aim at investigating the role of presynaptic adenosine receptors in mesenteric vessels and establish a relationship between their effects (in mesenteric vessels) and hypertension, using the spontaneously hypertensive rats (SHR) as a model of hypertension. Adenosine receptor-mediated modulation of noradrenaline release was investigated through the effects of selective agonists and antagonists on electrically-evoked [$^3$H]-noradrenaline overflow. CPA ($A_1$AR selective agonist: 1–100 nM) inhibited tritium overflow, but the inhibition was lower in SHR mesenteric vessels. IB-MECA ($A_3$AR selective agonist: 1–100 nM) also inhibited tritium overflow but only in WKY mesenteric veins. CGS 21680 ($A_{2A}$AR selective agonist: up to 100 nM) failed to facilitate noradrenaline release in mesenteric veins, from both strains, but induced a similar facilitation in the mesenteric arteries. NECA (non-selective AR agonist: 1, 3 and 10 μM), in the presence of $A_1$ (DPCPX, 20 nM) and $A_3$ (MRS 1523, 1 μM) AR selective antagonists, failed to change tritium overflow. In summary, the modulatory effects mediated by presynaptic adenosine receptors were characterized, for the first time, in mesenteric vessels: a major inhibition exerted by the $A_1$ subtype in both vessels; a slight inhibition mediated by $A_3$ receptors in mesenteric vein; a facilitation mediated by $A_{2A}$ receptors only in mesenteric artery (from both strains). The less efficient prejunctional adenosine receptor mediated inhibitory effects can contribute to an increase of noradrenaline in the synaptic cleft (both in arteries and veins), which might conduce to increased vascular reactivity.



## 1. Introduction

The mesenteric circulation plays an important role in the maintenance of systemic blood pressure and regulation of tissue blood flow (Takenaga and Kawasaki, 1999). It is well established that elevated blood pressure is associated with enhanced sympathetic nerve activity in both human hypertension (Matsukawa et al., 1993) and spontaneously hypertensive rats (SHR) (Judy et al., 1976; Lundin et al., 1984). Nevertheless, the sympathetic neurons innervating arteries and veins differ in their location in the ganglia and in their electrophysiological properties (Browning et al., 1999) suggesting a differential sympathetic neural control of mesenteric arteries and veins. Sympathetic nerves innervating the splanchnic circulation are particularly important (King et al., 2007): arteries and veins from the mesenteric bed are densely innervated by sympathetic nerve endings and are crucial in the regulation of the peripheral resistance and capacitance, respectively (Greenway, 1983; Pang, 2001; Park et al., 2007; Rothe, 1983). For instance, an increase in mesenteric venomotor tone leads to an increase in venous return and cardiac output with a profound impact on overall hemodynamics (Greenway, 1983). Moreover, it is well accepted that veins are more sensitive than arteries to the vasoconstrictor effects of sympathetic nerve stimulation (Hottenstein and Kreulen, 1987; Luo et al., 2004; Park et al., 2007).

Adenosine is a potent regulator of vascular tone, exerting its effects either directly on vascular smooth muscle cells or through prejunctional modulation of perivascular sympathetic neurotransmission (Burnstock and Kennedy, 1986; Olsson and Pearson, 1990).

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Adenosine actions are mediated through activation of four G-protein coupled receptors: $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ (Fredholm et al., 2001). In the literature, evidence indicates that these receptors have a role under both physiological and pathophysiological conditions (Fredholm et al., 2001), namely in hypertension. For example, the prolonged infusion of 1,3-dipropyl-8-sulfophenylxanthine (DPSPX), a non-selective antagonist of adenosine receptors, causes an hypertensive state (Matias et al., 1991). Moreover, some studies revealed a diminished inhibition of noradrenaline release, mediated by $A_1$-adenosine receptors, in arteries from SHR, when compared with normotensive animals (caudal artery: Illes et al., 1989; mesenteric artery: Rocha-Pereira et al., 2009, 2010). However, to our knowledge, there is no data on the effects mediated by presynaptic adenosine receptors in venous territories. Some indirect data suggested a modulatory role for adenosine receptors in venous neurotransmitter release since ATP and adenosine were found to be equipotent in inhibiting neurogenic contractions of the rabbit and portal vein, possibly by activating adenosine receptors (Burnstock et al., 1984).

In the present work, we intend to address the putative role of presynaptic adenosine receptors in mesenteric veins, compare the effects observed in veins with those observed in mesenteric arteries and, if possible, establish a putative relationship between adenosine receptor-mediated effects in mesenteric circulation and hypertension, by carrying out experiments with SHR mesenteric vessels.

## 2. Materials and methods

### 2.1. Animals

Adult male (12 week-old) SHR and Wistar Kyoto (WKY) rats (250–370 g; Charles River, Barcelona, Spain) were used. Animals were kept under light/dark cycles of 12/12 h, 20–22 °C, and had free access to water and pellet food. Handling and care of animals were conducted according to the European Union guidelines for animal research (86/609/EEC; in agreement with the NIH guidelines) and Portuguese law (Portarias n° 1005/92 and n° 1131/97). Animals were killed by stunning followed by exsanguination. Two animals per experiment were used and the mesenteric arteries and veins were dissected out and cleaned of fat and connective tissues. Three tissue preparations (~7 mg) were obtained from each artery or vein.

### 2.2. [$^3$H]-noradrenaline release experiments

After dissection, mesenteric arteries and veins were immediately placed in cold Krebs-Henseleit solution of the following composition (in mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaH_2PO_4$ 1.2, $NaHCO_3$ 25, glucose 11, ascorbic acid 0.3 and dissodium EDTA 0.03 (pH 7.4).

The protocol used to label tissue preparations with [$^3$H]-noradrenaline and to evaluate changes on electrically evoked tritium overflow was performed according to previous studies (Diniz et al., 2004; Fresco et al., 2002, 2007), with minor modifications. Briefly, mesenteric arteries and veins were pre-incubated in 2 ml Krebs-Henseleit solution containing 0.1 μM [$^3$H]-noradrenaline, for 40 min at 37 °C. Mesenteric arteries and veins were kept at 37 °C and continuously gassed with 95% $O_2$ and 5% $CO_2$. Desipramine (400 nM; to inhibit neuronal uptake of noradrenaline) was added at the onset of superfusion and kept throughout the experiment. Individual preparations were transferred into superfusion chambers between platinum electrodes and superfused with [$^3$H]-noradrenaline-free medium (1 ml min$^{-1}$; constant rate). A Stimulator II (Hugo Sachs Elektronik, March-Hugstetten, Germany), operating in a constant current mode, was used for electrical stimulation of the peri-arterial and peri-venous nerves: up to five periods of electrical stimulation, 2 Hz, 80 mA, 1 ms, 200 pulses. The first stimulation period, $t = 45$ min ($S_0$), served to test the viability of the preparation and medium was not collected during this period. The subsequent four stimulation periods ($S_1$–$S_4$) started at $t = 90$ min and were applied with 20 min intervals. The superfusate was collected in 5 min periods from 85 min of superfusion onwards ($t = 0$ min being the onset of superfusion). At the end of the experiments ($t = 170$ min), tritium was measured in superfusate samples and solubilized arteries and veins by liquid scintillation spectrometry (LS 6500, Beckman Instruments, Fullerton, USA) after adding 6 ml of scintillation mixture (OptiPhase 'Hisafe' 3, PerkinElmer, I.L.C., Lisboa, Portugal) to each superfusate sample or 2.5 ml perchloric acid (0.2 M) and 6 ml of scintillation mixture to each tissue.

From each animal, no more than two tissue preparations were submitted to identical treatments.

### 2.3. Data analysis

Evaluation of [$^3$H]-noradrenaline release experiments was carried out as previously described (Diniz et al., 2004; Fresco et al., 2002, 2007). Briefly, effects of drugs added after $S_1$ on electrically-evoked tritium overflow were evaluated as ratios of the overflow elicited by $S_n$ and the overflow elicited by $S_1$ ($S_n/S_1$). $S_n/S_1$ ratios obtained in individual experiments in which a test compound A was added after $S_1$ were calculated as a percentage of the respective mean ratio in the appropriate control group (solvent instead of A). When the interaction of A, added after $S_1$, and a drug B, added 5 min before $S_n$, was studied, the "appropriate control" was a group in which A alone was used.

Results are expressed as mean ± s.e.m. and *n* denotes the number of tissue preparations. Differences of means were compared for significance using one way ANOVA followed by *post hoc* Holm-Sidak's multicomparisons or unpaired Student's *t*-test. A P value less than 0.05 was considered to denote statistically significant differences.

### 2.4. Materials

The following drugs were used: levo-[ring-2,5,6-$^3$H]-noradrenaline, specific activity 49.5 or 42.5 Ci/mmol, was from DuPont NEN (I.L.C., Lisboa, Portugal); desipramine hydrochloride (uptake-1 inhibitor), $N^6$-cyclopentyladenosine (CPA: selective $A_1$ adenosine receptor agonist), 2-p-(2-carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine hydrochloride (CGS 21680: selective $A_{2A}$ adenosine receptor agonist), 1-deoxy-1-[6-[[(3-iodophenyl)-methyl]amino]-9H-purin-9-yl]-N-methyl-β-D-ribofuranuronamide (IB-MECA: selective $A_3$ adenosine receptor agonist), N-ethylcarboxamidoadenosine (NECA: non selective adenosine receptor agonist), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX: selective $A_1$ adenosine receptor antagonist), 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine (SCH 58261: selective $A_{2A}$ adenosine receptor antagonist), 2,3-diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarboxylate-5-carboxylate (MRS 1523: selective $A_3$ adenosine receptor antagonist), yohimbine hydrochloride ($\alpha_2$-adrenoceptor antagonist) were from Sigma–Aldrich (Sintra, Portugal).

Other reagents were of analytical grade. Stock solutions were made up in dimethylsulphoxide (DMSO: 0.01% v/v, final concentration) or distilled water and diluted in superfusion medium immediately before use. DMSO was added to the superfusion medium (final concentration 0.01%), in parallel control experiments.

## 3. Results

The fractional rate of basal tritium outflow ($b_1$) and electrically-evoked tritium overflow ($S_1$) of mesenteric vessels (artery and