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Nitric oxide synthase/K^+ channel cascade triggers the adenosine A_{2B} receptor-sensitive renal vasodilation in female rats

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ABSTRACT

Adenosine A_{2B}-receptors mediate the adenosine-evoked renal vasodilations in male rats. Here, we tested whether this finding could be replicated in female renal vasculature and whether K^+ hyperpolarization induced by nitric oxide synthase (NOS) and/or heme oxygenase (HO) accounts for adenosine A_{2B} receptor-sensitive renal vasodilations. In phenylephrine-preconstricted perfused kidneys, vasodilations caused by the adenosine analog 5′-*N*-ethylcarboxamidoadenosine (NECA, 1.6–50 nmol) were attenuated after blockade of adenosine A_{2B} (alloxazine) but not A_{2A} [8-(3-Chlorostyryl) caffeine, CSC] or A_3 receptors (N-(2-methoxyphenyl)-N′-[2-(3-pyridinyl)-4-quinazolinyl]-urea, VUF 5574), confirming the preferential involvement of A_{2B} receptors in NECA responses. NOS activation mediated the A_{2B} receptor-mediated NECA response because: (i) NOS inhibition (N^{ω}-nitro-L-arginine-methyl ester, L-NAME) attenuated NECA vasodilations, (ii) concurrent L-NAME/alloxazine exposure caused more inhibition of NECA responses, and (iii) inhibition of NECA responses by alloxazine disappeared in L-arginine-supplemented preparations. Although HO inhibition (zinc protoporphyrin) failed to modify NECA responses, the attenuation of these responses by alloxazine disappeared in hemin (HO inducer)-treated preparations. NECA vasodilations were also attenuated after exposure to $BaCl_2$, glibenclamide but not tetraethylammonium (blockers of inward rectifier, ATP-sensitive, and Ca^{2+}-dependent K^+-channels, respectively). The combined alloxazine/$BaCl_2$/glibenclamide infusion caused no additional attenuation of NECA vasodilations. Vasodilations caused by minoxidil (K^+-channel opener) were reduced by L-NAME or $BaCl_2$/glibenclamide, supporting the importance of NOS signaling in K^+ hyperpolarization. NECA or minoxidil vasodilations were attenuated by ouabain, Na^+/K^+-ATPase inhibitor, and in KCl-preconstricted preparations. Overall, facilitation of adenosine A_{2B} receptor/NOS/K^+ channel/Na^+/K^+-ATPase cascade underlies NECA vasodilations in female rats. Enhancing HO activity, albeit not causally related to NECA vasodilations, improves the pharmacologically compromised (alloxazine) NECA response.

1. Introduction

Four adenosine receptor subtypes have been cloned in the kidney and designated as A_1, A_{2A}, A_{2B}, and A_3 receptors (Vallon and Osswald, 2009). Whereas adenosine A_1 receptors mediate renal vasoconstriction via decreasing NO generation and increasing the vasoconstrictor products of the COX pathway (Barrett and Droppleman, 1993; Walkowska et al., 2007), A_{2A} and A_{2B} receptors are involved in the vasodilatory effect of adenosine probably via stimulation of NO and epoxyeicosatrienoic acid production (Rekik et al., 2002; Carroll et al., 2006; Feng and Navar, 2010). While both adenosine A_{2A} and A_{2B} receptors are functionally expressed in renal afferent arterioles, recent evidence suggests that the renal vasodilatory action of adenosine is mediated predominantly via the adenosine A_{2B} receptor activation (Feng and Navar, 2010). Alternatively, reported studies on the modulatory role of adenosine A_3 receptors on vascular control are contradictory (Hinschen et al., 2003; Ansari et al., 2007a).

Potassium conductance is a major determinant of membrane potential in vascular smooth muscle and endothelial cells. Whereas increased K^+ conductance leads to hyperpolarization and vasodilation, inactivation of K^+ channels causes depolarization and vasoconstriction (Sorensen et al., 2012). In the coronary microcirculation, the activation of the NOS/ATP-sensitive K^+ channel pathway mediates the adenosine A_{2A} receptor-dependent coronary vasodilation (Hein et al., 1999; Sanjani et al., 2011). Others implicated both the voltage- and ATP-sensitive K^+ channels in vasodilatory responses elicited by the activation of adenosine A_2 receptors (Berwick et al., 2010). Like NO, accumulating evidence underscores a vasodilatory role of carbon

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monoxide (CO) that is both NO/cGMP and K^+ channel-dependent. In support of this, pharmacological inhibition of HO leads to a reduction in CO production and increases renal vascular resistance (Kozma et al., 1999; Lamon et al., 2009). CO promotes relaxation in resistance vessels by stimulating calcium-activated K^+ channels (Wang and Wu, 1997; Kaide et al., 2001). Moreover, the activation of HO/CO signaling mediates some of the biological effects of adenosine. For example, a positive feedback loop exists between HO/CO and adenosine A_{2A} receptors in the inflammatory response in macrophages (Haschemi et al., 2007). Also, in medullary neurons of the brainstem, ARs blockade or HO inhibition attenuates the hypotensive response elicited by hemin (HO inducer) and adenosine, respectively, suggesting the presence of crosstalk between adenosinergic and HO pathways (Lin et al., 2003).

The goal of the current investigation was twofold. First, because earlier studies that implicated renal adenosine A_{2B} receptors in the vasodilatory effect of adenosine was performed in male rats (Feng and Navar, 2010), we thought it was important to determine whether this phenomenon could be replicated in the female population. Remarkably, evidence highlights important roles for gender and hormonal factors in the regulation of vascular tone (Thompson and Khalil, 2003) and suggests sexual dimorphism in the renovascular responsiveness to nicotine and in the nicotine-β-adrenoceptor interaction in the renal vasculature (El-Mas et al., 2009, 2011). The second, and more important, objective of the current study was to test the hypothesis that facilitation of the NO/CO/K^+ channel cascade constitutes the cellular mechanism that underlies the adenosine A_{2B} receptor-sensitive vasodilatory action of NECA. The NECA response was evaluated in the presence of pharmacologic manipulations that (i) alter (inhibit or facilitate) NOS or HO activities, (ii) block the inward rectifier, ATP-sensitive, or Ca^{2+}-dependent K^+ channels, and (iii) inhibit Na^+/K^+-ATPase. To further consolidate our hypothesis, the effects of these pharmacologic interventions on renal vasodilations caused by the K^+ channel opener minoxidil were also investigated. The contribution of K^+ channels as downstream effectors to the vasodilatory response elicited by the activation of adenosine receptors (Tang et al., 1999; Berwick et al., 2010), NO (Hein et al., 1999; Sanjani et al., 2011), or CO (Wang and Wu, 1997; Kaide et al., 2001) has been documented in renal and non-renal vascular preparations.

2. Materials and methods

Female Wistar rats (200–240 g; Faculty of Pharmacy, Alexandria University, Egypt) were used in the present study. Experiments were performed in strict accordance with institutional guidelines.

2.1. The rat isolated perfused kidney

The rat kidney was isolated and perfused according to the method described in our previous studies (El-Mas et al., 2004a; Abd-Elrahman et al., 2010; El-gowelli et al., 2011). Briefly, rats were anesthetized with thiopental sodium (50 mg/kg, i.p.), the abdomen was opened by a midline incision, and the left kidney was exposed. The left renal artery was dissected free from its surrounding tissues. Loose ties were made around the renal artery and the abdominal aorta, proximal and distal to the renal artery. A beveled 18-gauge needle connected to a 5 mL syringe filled with heparinized saline (100 U/ml) was used for cannulation. The aorta was ligated, and the left renal artery was cannulated via an incision made in the aorta. The cannula was immediately secured with ligatures, and the kidney was flushed with heparinized saline and rapidly excised from its surrounding tissues.

The kidney was transferred into a jacketed glass chamber maintained at 37 °C and continuously perfused with Krebs solution (in mM: NaCl, 120; KCl, 5; $CaCl_2$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11) maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Kidney perfusion was adjusted at a constant flow rate of 5 ml/min using a peristaltic pump (Master Flex® L/S, Barnant company, USA), which achieves perfusion pressures that were compatible with adequate kidney function (Dobrowolski et al., 1998). The pump delivered a pulsatile flow, and an open circuit was used so that the venous effluent was allowed to drain freely. The kidney perfusion pressure was continuously monitored by means of a MLT844 physiological pressure transducer (AD instruments, Australia) distal to the pump and recorded on Power Lab 4/35 data acquisition system using LabChart Pro 7 software (AD instruments, Australia). Inasmuch as the renal flow was kept constant, changes in perfusion pressure were indicative of alterations in renal vascular resistance. An equilibration period of 30 min was allowed at the beginning of the experiment to ensure stabilization of the kidney perfusion pressure. To study the vasodilatory effects of NECA or minoxidil, the renal vascular tone was elevated by continuous infusion of the α_1-adrenoceptor agonist phenylephrine (20 μM). The infusion of phenylephrine into the renal vasculature produced an abrupt increase in perfusion pressure, which was stabilized within 10–20 min for the remainder of the experiment (El-Mas et al., 2003, 2005).

2.2. Protocols and experimental groups

2.2.1. Effect of selective blockade of adenosine receptor subtypes on NECA vasodilations

This experiment investigated the relative contributions of adenosine receptor subtypes (A_{2A}, A_{2B}, and A_3) in renal vasodilations induced by NECA in phenylephrine-preconstricted perfused kidney. Four groups (n=6–8 each) of female Wister rats were employed to determine the effects of CSC (A_{2A} receptor blocker), alloxazine (A_{2B} receptor blocker), or VUF 5574 (A_3 receptor blocker) on the evoked renal vasodilations. The vasodilatory responses of the renal vasculature to cumulative bolus injections of NECA (1.6–50 nmol) were established and changes in the renal perfusion pressure were monitored. This was followed by continuous infusion of CSC (0.5 μM; Grbović et al., 2000), alloxazine (10 or 60 μM; Ansari et al., 2007b, Li et al., 2007), VUF 5574 (0.1 μM; Jackson et al., 2011), or the vehicle dimethyl sulfoxide (DMSO). 20 min later, cumulative dose-vasodilatory response curves for NECA were re-established.

2.2.2. Role of NOS or HO signaling in NECA-alloxazine renal interaction

Because data obtained from the preceding experiment selectively implicated adenosine A_{2B} receptors in the vasodilatory action of NECA, in this experiment we tested the hypothesis that the adenosine A_{2B} receptor-sensitive NECA vasodilation is modulated by NOS/NO and/or HO/CO signaling pathways. Six groups of rats (n=6–7 each) were used to test the effects of pharmacologic maneuvers that inhibit or facilitate the activity of NOS (L-NAME and L-arginine, respectively) or HO (zinc protoporphyrin, ZnPP, and hemin, respectively) on NECA responses in the absence or presence of alloxazine. Cumulative dose-response curves of NECA (1.6–50 nmol) were established in phenylephrine-preconstricted kidneys before (control) and 20 min after the infusion of one of the following regimens: (i) L-NAME (100 μM; El-Gowelli et al., 2011), (ii) L-NAME + alloxazine (10 μM), (iii) L-arginine (100 μM;