Role of Adenosine Receptor(s) in the Control of Vascular Tone in the Mouse Pudendal Artery

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ABSTRACT

Activation of adenosine receptors (ARs) has been implicated in the modulation of renal and cardiovascular systems, as well as erectile functions. Recent studies suggest that adenosine-mediated regulation of erectile function is mainly mediated through A2AAR activation. However, no studies have been conducted to determine the contribution of AR subtype in the regulation of the vascular tone of the pudendal artery (PA), the major artery supplying and controlling blood flow to the penis. Our aim was to characterize the contribution of AR subtypes and identify signaling mechanisms involved in adenosine-mediated vascular tone regulation in the PA. We used a DMT wire myograph for muscle tension measurements in isolated PAs from wild-type, A2AR knockout, A2AR knockout, and A2A/A2AR double-knockout mice. Real-time reverse transcription–polymerase chain reaction was used to determine the expression of the AR subtypes. Data from our pharmacologic and genetic approaches suggest that AR activation–mediated vasodilation in the PA is mediated by both the A2AAR and A2AR, whereas neither the A1AR nor A3AR play a role in vascular tone regulation of the PA. In addition, we showed that A2AAR- and A2AR-mediated vasorelaxation requires activation of nitric oxide and potassium channels; however, only the A2AAR-mediated response requires protein kinase A activation. Our data are complemented by mRNA expression showing the expression of all AR subtypes with the exception of the A3AR. AR signaling in the PA may play an important role in mediating erection and represent a promising therapeutic option for the treatment of erectile dysfunction.

Introduction

Penile erection consists of multiple neurovascular processes that all simultaneously involve the nerves, blood vessels, and endothelium in the sinusoids and trabecular smooth muscle cells of the penis. Those factors, which regulate contraction and relaxation of vascular smooth muscle, determine the state of the penis (flaccidity versus erection) (Andersson, 2001; Tostes et al., 2007; Nunes et al., 2012). The flaccid state is mainly mediated by the release of norepinephrine from adrenergic nerve terminals and other vasoconstrictors, such as endothelin-1 and angiotensin II (Sáenz de Tejada et al., 1989, 2004). The erection is mainly mediated by nitric oxide (NO) released from the endothelium and nonadrenergic-noncholinergic nerves in addition to other neurotransmitters and endothelium-derived hyperpolarizing factors (EDHFs), such as adenosine (Chiang et al., 1994; Tostes et al., 2007; Wen et al., 2011b).

Adenosine, a signaling nucleoside, is produced during conditions of metabolic stress and high cellular activity, resulting in increased oxygen supply and decreased oxygen consumption. Adenosine is mainly generated by the 5′-nucleotidases CD73 that catalyze the dephosphorylation of AMP into adenosine. Intracellular adenosine levels are primarily regulated by adenosine kinase, which converts adenosine to AMP, whereas extracellular adenosine levels are critically regulated by adenosine deaminase, which degrades adenosine to inosine (Blackburn, 2003; Ham and Evans, 2012; Wen and Xia (2012)). Recently, adenosine was described as an EDHF because of its ability to relax and hyperpolarize vascular smooth muscle cells (VSMC) (Ohta et al., 2013). The activation of adenosine receptors (ARs) is implicated in the modulation of renal and cardiovascular functions, as well as erectile function, with both in vivo and in vitro studies demonstrating that, like NO, adenosine is a potent vasodilator.
that may regulate penile erection in humans and animals (Takahashi et al., 1991, 1992; Chiang et al., 1994; Mantelli et al., 1995; Filippi et al., 2000; Noto et al., 2001; Tostes et al., 2007; Carneiro et al., 2008; Mi et al., 2008; Vallon and Osswald, 2009; Phatarpekar et al., 2010; Wen et al., 2010, 2011b, 2012; Ning et al., 2012; Headrick et al., 2013; Layland et al., 2014). Adenosine binds to a family of four P1 G protein-coupled AR subtypes: A1, A2A, A2B, and A3. Vascular studies from our laboratory and others have demonstrated that, whereas the activation of the A1AR and A3AR results in vasoconstriction, the activation of the A2AAR and A2BAR mediates the CC relaxation. Studies in humans and animals reported that, both the A2AAR and A2BAR mediate the CC’s vasorelaxation (Filippi et al., 2000; Noto et al., 2001; Faria et al., 2006; Tostes et al., 2007; Mi et al., 2008; Moura et al., 2015). Using quantitative real-time polymerase chain reaction (qPCR), Mi et al. (2008) showed that the A2BAR was the predominant receptor subtype expressed in murine cavernosal smooth muscle cells, whereas relatively low level expression of the A2AAR was observed. The subtypes promoting vasoconstriction, namely the A1AR and A3AR, were not detectable. In contrast, an earlier study by Tostes et al. (2007) suggested that activation of both the A2AAR and A2BAR mediate CC relaxation in mice. While the contribution of the A1AR to erectile function plays an important role in the release of neurotransmitters, the role of the A3AR in erectile function is still not known (Chiang et al., 1994; Tostes et al., 2007; Ning et al., 2012). Recent studies demonstrated that adenosine functions to promote penile erection; however, this research has focused solely on the CC.

In addition to the CC, an important player in regulating erectile function and blood flow to the penis is the pudendal artery (PA). The PA is an artery that branches from the internal iliac artery, providing oxygenated blood to the external genitals. The PA branches into cavernous arteries that further branch into tortuous helicine arteries, all feeding the cavernous sinuses. In the PA, the absence of capillaries allows for a rapid filling of cavernosal sinuses during erection (Hale et al., 2009). Recent studies have shown that the PA contributes approximately 70% of total pudendal-penile vascular resistance, whereas the intrapenile vasculature contributes less than 25% of total resistance in this bed (Manabe et al., 2000). In addition, it has been demonstrated that optimal erection requires vasodilation of both prepenile arteries, such as the PA, and as well as intrapenile arteries (Manabe et al., 2000; Hale et al., 2009; Hannan et al., 2011). To date, no studies have characterized the contribution of adenosine and its receptors in the regulation of the PA vascular tone. In the present study, we characterized the contribution of AR subtypes to vascular tone in the PA and identified signaling mechanisms involved in adenosine-mediated vascular tone regulation.

Materials and Methods

Animals. The Institutional Animal Care and Use Committee of West Virginia University approved all experimental protocols. We followed guidelines set forth by the American Physiologic Society and National Institutes of Health regarding the care and use of laboratory animals. A2AR and A3AR single knockout (KO) mice (A2AR KO and A3AR KO mice, respectively) were generously provided by Dr. C. Ledent (Universite libre de Bruxelles, Brussels, Belgium) and Stephen Tilley (University of North Carolina, Chapel Hill, NC), respectively. A2AR and A3AR KO mice, both backcrossed 12 generations to wild-type (WT) C57Bl/6 background mice (Jackson Laboratory, Bar Harbor, ME) were bred to generate A2AAR/A2BAR double-KO (DKO) heterozygotes. Double heterozygotes were intercrossed, and 1/16 of the offspring were A2AAR/A2BAR DKO mice. A2AAR/A2BAR DKO breeding pairs were then established (Zhou et al., 2014). Mice were caged on a 12-hour light/dark cycle with free access to standard chow diet, with water ad libitum.

Muscle Tension Studies in Pudendal Arteries. Mice were euthanized using sodium pentobarbital (50 mg/kg i.p.). PAs were excised, transferred into an ice-cold physiologic salt solution (130 mM NaCl, 14.9 mM NaHCO3, 5.5 mM dextrose, 1.8 mM KH2PO4, 1.17 mM MgSO4 · 7H2O, 1.6 mM CaCl2 · 2H2O), and dissected to remove loose connective tissue and fat. The arteries were then cut into 2-mm segments and mounted on a wire myograph in 5-ml chambers (DMT, Aarhus, Denmark) containing buffer at 37°C and continuously bubbled with a mixture of 95% O2 and 5% CO2. The tissues were stretched to a resting force of 200 mg and allowed to equilibrate for 60 minutes. Changes in isometric force were recorded using a PowerLab/SP data acquisition system (Chart software, version 5.0; ADInstruments, Colorado Springs, CO). After equilibration, rings were precontracted with 50 mM KCl to check the contractility of the individual PA rings. Arterial and endothelial integrity were assessed by contracting with phenylephrine (Phe, 10−3 M), followed by relaxation with acetylcholine (ACh, 10−4 M). Concentration-response curves (CRC) for AR agonists [5′-N-ethylcarboxamidoadenosine (NECA), 2-chloro-N6-cyclopentyladenosine (CCPA), and 1-[2-chloro-6-[[3-isodophenyl)methyl]amino]-9H-purin-9-yl-1-deoxy-N-methyl-beta-d-ribofuranuronamide (CI-IBMECA), 10−8 M to 10−2 M] were performed. Endothelium-dependent relaxation was assessed by measuring the dilatatory response to ACh (10−4 M to 10−2 M) in Phe-contracted vessels.

Drugs and Solutions. ACh, Phe, NECA, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; an A1AR antagonist), KT 5720 [a protein kinase A (PKA) inhibitor], MRS1523 (an A2AR antagonist), N6-nitro-arginine methyl ester [L-NAME; a nitric oxide synthase (NOS) inhibitor], and CI-IBMECA (an A2AR agonist) were purchased from Sigma-Aldrich/MilliporeSigma (St. Louis, MO). CCPA (an A1AR antagonist), SCH-58261 (a selective A2AAR antagonist), and CVT-6883 (a selective A3AR antagonist) were purchased from Tocris Bioscience (Minneapolis, MN). Stock solutions were prepared in deionized water or dimethyl sulfoxide (DMSO) and stored in aliquots at −20°C. Dilutions were prepared immediately before use. Rings were preincubated with antagonist or inhibitors 30 minutes prior to Phe precontraction for the CRC. Stock solutions originally diluted in DMSO were used with a final concentration of less than 0.003% v/v in the muscle bath; this concentration has been demonstrated to have no effect on vascular reactivity.

Real-Time Reverse Transcription–Polymerase Chain Reaction. Total RNA was isolated from PAs of WT mice using an RNAEasy Total RNA isolation kit from Qiagen (Valencia, CA). This was followed by conversion of 0.5 μg of total RNA into complementary DNA (cDNA) using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) according to manufacturer’s instructions in a total volume of 100 μl. Each sample contained PAs pooled from seven to nine mice. Because of the relatively low expression of ARs, a polymerase chain reaction (PCR) PreAmplification Kit (Applied Biosystems) was used. Reverse transcription–polymerase chain reaction (qPCR) was performed using an ABI PRISM 7300 Detection System (Applied Biosystems) with TaqMan Universal Master Mix (Applied Biosystems, Branchburg, NJ) according to manufacturer’s instructions. The reaction volume (25 μl) included...
12.5 μl of 2× TaqMan Universal Master Mix, 1 μl of cDNA, and 1.25 μl of 20× FAM-labeled TaqMan Gene Expression Assay Master Mix solution. For real-time PCR of AR genes, the TaqMan-inventoried gene expression product was purchased from Applied Biosystems. The 18S ribosomal RNA was used as an endogenous control. The fold-difference in expression of target cDNA was determined using the comparative CT method. The ΔCT value was determined in each experiment by subtracting the average 18S CT value from the corresponding average CT for the A1, A2A, A2B, and A3AR in coronary arteries, as previously described (Teng et al., 2013).

**Statistical Analysis.** Student’s t test was used for the comparison between two groups, and one-way analysis of variance was used for groups of three or more. CRC data were analyzed between groups at the same concentrations. In addition, an F-test was used for the estimation of EC50 values obtained from best-fit analysis using a nonlinear, interactive fitting program (GraphPad Prism; Graph Pad Software Inc. San Diego CA). Data are expressed as means ± S.E.M. (N), where N is the number of mice. Values of P < 0.05 were considered a statistically significant difference.

**Results**

**NECA-Mediated Relaxation in the PA Is Dependent on A2AAR and A2BAR Activation.** In preconstricted PA rings isolated from WT mice, NECA (a nonselective AR agonist) produced a concentration-dependent relaxation, suggesting a role for either the A2AAR or A2BAR, since both ARs are associated with vascular relaxation. Preincubation with either the A2AAR antagonist SCH-58261 or A2BAR antagonist CVT-6883 significantly decreased the NECA-mediated relaxation (Fig. 1A). Furthermore, PAs isolated from A2AAR KO mice exhibited a decreased relaxation response to NECA (EC50 = 5.88 ± 0.10 in WT versus −5.40 ± 0.17 in A2AAR KO mice, P < 0.05) (Fig. 1B). Incubation of PAs isolated from A2AAR KO mice with the A2BAR antagonist abolished the NECA-mediated vasorelaxation (Fig. 1B). Contrary to A2AAR KO mice, PAs isolated from A2BAR KO mice exhibited an increase in sensitivity to NECA compared with PAs from WT mice (−5.88 ± 0.10 versus −6.50 ± 0.15, P < 0.05) (Fig. 1C). Incubation of PAs isolated from A2BAR KO mice with the A2AAR antagonist abolished the NECA-mediated vasorelaxation (Fig. 1C). Furthermore, genetic deletion of both the A2AAR and A2BAR resulted in the absence of vasodilation in response to increased NECA concentrations (Fig. 1D). Taken together, our data suggest that both the A2AAR and A2BAR contribute to relaxation in the PA.

**Neither A1AR Nor A3AR Activation Resulted in Vasorelaxation in the PA.** Activation of either A1AR or A3AR is known to result in vasorelaxation. Cumulative addition of CCPA (an A1AR agonist) or Cl-BMECA (an A3AR agonist) did not cause vasoconstriction in PAs isolated from WT mice. Furthermore, both CCPA and Cl-BMECA caused vasorelaxation of PAs at high concentrations (10−6 M and 10−5 M), possibly owing to nonselectivity of the agonists; however, the vasodilatory response to CCPA and Cl-BMECA was significantly lower in comparison with that elicited by NECA (Fig. 2).

**Neither A1AR nor A3AR Is Required for AR-Mediated Vasorelaxation.** Since A1AR or A3AR agonists were shown to activate either the A2AAR or A2BAR at high concentrations, we used PAs isolated from A2A/A2BAR DKO mice (Fig. 3). In preconstricted PAs isolated from A2A/A2BAR DKO mice, NECA (Fig. 2) did not cause relaxation, whereas both CCPA and Cl-BMECA caused dilation at the highest dose (10−5 M) (Fig. 3A). Incubation of PAs isolated from A2A/A2BAR DKO mice with both the A1AR antagonist DPCPX and the A3AR antagonist MRS-1593 did not affect CCPA- and Cl-BMECA-mediated relaxation at the 10−5 M concentration, suggesting that the dilation mediated by CCPA and Cl-BMECA at the 10−5 M concentration is not dependent on ARs (Fig. 3, B and C).

**Genetic Deletion of A2AAR or A2BAR Resulted in Decreased Endothelium-Mediated Vasorelaxation.** Recently, adenosine was described as an EDHF owing to its ability to activate either the A2AAR or A2BAR at high concentrations, to cause increased NECA concentrations (Fig. 1D). Taken together, our data suggest that both the A2AAR and A2BAR contribute to relaxation in the PA.
to relax and hyperpolarize VSMCs. To test the effect of either A2AAR or A2BAR deletion on the endothelium-mediated dilation, increased concentration of ACh resulted in a concentration-dependent relaxation in PAs isolated from WT, A2AAR KO, and A2BAR KO mice (Fig. 4A). However, PAs isolated from A2A KO or A2B KO mice exhibited a decrease in EC50 in response to ACh compared with WT mice: EC50 –7.78 ± 0.06 in WT versus –7.29 ± 0.06 in A2A KO, P < 0.05; and EC50 –7.78 ± 0.06 in WT versus –7.26 ± 0.10 in A2B KO, P < 0.05 (Fig. 4B). Taken together, our data suggest that both the A2AAR and A2BAR may play a role in the endothelium-mediated relaxation.

**Mechanism of A2AAR- and A2BAR-Mediated Relaxation in the PA.** To look at the mechanisms of A2AAR-mediated relaxation, we used PAs isolated from A2B KO mice (Fig. 5A). Preincubation with either an NOS inhibitor (L-NAME), protein kinase A inhibitor (KT 5720), or potassium channel inhibition (5 mM KCl) significantly decreased A2A-mediated vasorelaxation in PAs isolated from A2A KO mice. To elucidate the mechanisms of A2BAR-mediated relaxation, we used PAs isolated from A2A KO mice (Fig. 5B). Pretreatment with the either L-NAME or increased extracellular K+ significantly decreased the A2BAR-mediated vasorelaxation, whereas treatment with KT 5720 did not affect relaxation in PAs isolated from A2A KO mice. Taken together, our data suggest that both A2AAR- and A2BAR-mediated vasorelaxation requires activation of NO and K+ channels; however, only the A2AAR-mediated response requires PKA activation.

**Message RNA Expression of ARs in the PA.** In support of our observations made in the functional studies, semiquantitative real-time PCR was used to determine mRNA expression of the AR subtypes in PAs isolated from WT mice. Of the four AR subtypes, only the A1R, A2AAR, and A2BAR were expressed, with the A2AAR having the highest mRNA expression level compared with the A1R and A2BAR (Fig. 6).

**Discussion**

Using pharmacological and genetic approaches, our study is the first to investigate transcriptional expression and to characterize the contribution of the four AR subtypes to...
vascular tone regulation of the PA. Herein, we showed that: 1) of the four AR subtypes, only the A1R, A2AAR, and A2BAR were expressed in the PA; 2) although the A1AR was expressed in the PA, only the A2AAR and A2BAR played a role in vascular tone regulation of the PA; and 3) both A2AAR- and A2BAR-mediated vasorelaxation requires activation of NOS and K+ channels; however, only A2AAR-mediated responses requires PKA activation. In this paper, we sought to fill a gap in the understanding of AR signaling in erectile function and define the roles of AR subtypes in prepenile vascular tone regulation.

Numerous studies have investigated the contribution of adenosine to erectile function, looking at the effect of AR activation in the CC. However, this study is the first to characterize the contribution of ARs in the regulation of PA vascular tone, the major artery supplying and controlling blood flow to the penis (Manabe et al., 2000; Hale et al., 2009). In the first set of experiments, we observed that the adenosine analog NECA caused a concentration-dependent vasorelaxation in murine PAs. As we already know, A2AAR and A2BAR activation results in vasorelaxation, whereas A1AR and A3AR activation results in vasoconstriction. In this study, our results suggested that NECA-induced relaxation is mediated through A2AAR and A2BAR activation. Indeed, preincubation of PA rings with specific A2AAR (SCH-58261) or A2BAR (CVT-6883) antagonists significantly decreased the NECA-mediated relaxation, suggesting that both A2AAR and A2BAR activation are required for the response to NECA in PAs (Fig. 1A). Furthermore, PAs isolated from A2A KO mice exhibited a decreased relaxation to NECA, which was abolished using an A2BAR antagonist. Contrary to A2A KO mice, PAs isolated from A2B KO mice exhibited an increase in sensitivity to NECA compared with PAs from WT mice; however, when incubated with an A2AAR antagonist, the relaxation response to NECA was completely abolished. The difference in PA responses using pharmacological inhibition, compared with other genetic KO models, can be explained by the fact that global gene deletion usually results in compensatory upregulation of other genes or other compensatory signaling pathways. Regarding A2A and A2B ARs, although it has been shown that a single gene KO results in upregulation of the other AR in mesenteric artery (Teng et al., 2013), this was not the case in aorta, where KO of A2AAR gene did not affect the expression of A2BAR (Ponnoth et al., 2009). This also confirms the importance of pharmacological studies in addition to studies using gene deletion. In addition, the differences in the affinity of the receptors to the agonist could also explain the different response to NECA in the PA isolated from the KO mice, since A2BAR possesses the lowest affinity for adenosine and NECA. Our results were confirmed using PAs isolated from A2A/A2BAR DKO mice, where NECA failed to cause vasorelaxation in the PA rings. Our data confirm the important role played by both the A2AAR and A2BAR in CC relaxation and, thus, erection. In fact, several studies reported the role of both the A2AAR and A2BAR in mediating CC vasorelaxation (Filippi et al., 2000; Noto et al., 2001; Faria et al., 2006; Tostes et al., 2007). In mice, a recent study using a genetic approach by Mi and colleagues (2008) showed high

![Fig. 4.](image1)

![Fig. 5.](image2)
A2BAR expression in the CC compared with expression of the A2AAR. They also showed that vasorelaxation of the CC is mediated solely through the activation of the A2BAR, whereas the A2AAR did not seem to play a role. However, Tostes et al. (2007) showed that both A2AAR antagonist SCH-58261 and A2BAR antagonist MRS-1706 resulted in decreased CC relaxation to the nonspecific agonist 2-chloroadenosine, with the combination of both antagonists resulting in almost total inhibition of the CC’s relaxation, supporting our data in the present study, whereas both the A2AAR and A2BAR contribute to relaxation of the CC. In addition, other groups also have shown the contribution of the A2AAR to vasorelaxation in the CC (Mantelli et al., 1995; Filippi et al., 2000; Noto et al., 2001; Faria et al., 2006; Moura et al., 2015).

A1AR and A2AAR activation has been shown to mediate vascular contraction (Ansari et al., 2007; El-Awady et al., 2011; Kunduri et al., 2013). To test the contribution of the A1AR and A2AAR in the regulation of PA vascular tone, we performed CRCs to both an A1AR agonist (CCPA) and A2AAR agonist (Cl-IBMECA). Both antagonists failed to mediate vasoconstriction in PAs, suggesting that neither A1AR nor A2AAR contribute to the vascular tone in PAs. However, both drugs at high concentrations caused PA relaxation. These results were not surprising, as both drugs at high concentrations activate other receptors, including A2AAR and A2BAR (Teng and Mustafa, 2011). To further rule out the role of A1AR and A2AAR, we performed the CRC to an A1AR agonist (CCPA) and A2AAR agonist (Cl-IBMECA) in PAs isolated from A2AAR KO and A2BAR KO mice. To our surprise, both agonists (CCPA and Cl-IBMECA) were able to mediate vasorelaxation in PAs from A2AAR/KO and A2BAR/KO DKO mice. Our data suggest that A2AAR-mediated relaxation of the PA is dependent on PKA, NO, and K+ channel blockade, whereas A2BAR-mediated relaxation, we used PAs isolated from A2BAR KO mice to study A2AAR-mediated relaxation and A2AAR KO mice to study A2BAR-mediated relaxation in the PA (Fig. 5). Our data suggest that A2AAR-mediated relaxation in the PA is dependent on PKA, NO, and K+ channel activation. On the other hand, we found that A2BAR-mediated relaxation in the PA is dependent on NO and K+ channel activation, but not PKA, as PKA inhibition did not result in decreased NECA-mediated vasorelaxation in PAs isolated from A2AAR KO mice. In fact, a recent study showed that A2AAR contributed to penile erection via an alternative signaling pathway, which is dependent on PI3K/AKT signaling (Wen et al., 2011b).

Several studies have looked at the contribution of adenosine to erectile function both in vivo and in vitro. However, limited studies have tried to characterize AR expression in the erectile system. Using liquid chromatography–tandem mass spectrometry (LC-MS/MS), Mi et al. (2008) demonstrated that the A2BAR is the predominant receptor, with relatively low expression of the A2AAR, whereas mRNA levels of both the A1AR and A2AAR were nondetectable in CC smooth muscle cells. A similar pattern of AR expression was found in another study using primary CC fibroblast cells (Wen et al., 2011b). However, the expression level of ARs at the whole tissue level or other cell types in the CC (i.e., endothelial cells) was not evaluated. In the present study, we used isolated PAs to evaluate the expression pattern of AR subtypes using qPCR. We found that A1AR, A2AAR, and A2BAR were expressed in PAs (with higher expression of A2AAR compared with A1AR and A2BAR), whereas A2AAR mRNA levels were not detectable (Fig. 5A). Our data suggest that A2AAR-mediated relaxation in the PA is dependent on NO and K+ channel activation. On the other hand, we found that A2BAR-mediated relaxation in the PA is dependent on NO and K+ channel activation, but not PKA, as PKA inhibition did not result in decreased NECA-mediated vasorelaxation in PAs isolated from A2AAR KO mice. In fact, a recent study showed that A2AAR contributed to penile erection via an alternative signaling pathway, which is dependent on PI3K/AKT signaling (Wen et al., 2011b).

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and sildenafil (a PDE5 inhibitor), suggesting that combined agonist, since our study showed that both A2AAR and A2BAR will make the use of A2BAR agonist as a potential therapeutic hypertensive together with its association with penile fibrosis (Chiang et al., 1994; Kilic et al., 1994).

Xen and Xia (2012) suggested A2AR activation as a potential therapeutic pathway for ED; however, further studies looking at the change of AR expression in diseases associated with vasogenic ED, such as hypertension and diabetes, are crucial. In fact, using an angiotensin II model of hypertension, the same group demonstrated that in this model, which was also shown to be associated with ED, A2AR expression and signaling increased in the kidney, resulting in progression of hypertension associated with increased renal fibrosis (Jin et al., 2008). In addition to studies showing the involvement of A2AR signaling in mediating human CC relaxation. Furthermore, a recent study demonstrated a synergic effect of a novel A2AAR agonist and sildenafil (a PDE5 inhibitor), suggesting that combined treatment may reduce side effects and increase efficacy in ED patients who do not respond to sildenafil alone (Moura et al., 2015). Together, A2AR activation may represent an alternative clinical target for the treatment of ED, although further studies looking at the change of AR expression and signaling in diseases associated with vasogenic ED would be critical to evaluate the safety and efficacy of A2AAR agonists use in ED treatment.

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Authorship Contributions

Participated in research design: Labazi.

Conducted experiments: Labazi.

Contributed new reagents or analytic tools: Tilley, Ledent.

Performed data analysis: Labazi.

Wrote or contributed to the writing of the manuscript: Labazi, Mustafa.

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