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Mechanisms underlying uridine adenosine tetraphosphate-induced vascular contraction in mouse aorta: Role of thromboxane and purinergic receptors

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ABSTRACT

Uridine adenosine tetraphosphate (Up_4A), a novel endothelium-derived vasoactive agent, is proposed to play a role in cardiovascular disorders and induces aortic contraction through activation of cyclooxygenases (COXs). We and others demonstrated that activation of A_1 or A_3 adenosine receptors (ARs) results in vascular contraction via thromboxane (TX) A_2 production. However, the mechanisms of Up_4A-induced vascular contraction in mouse aorta are not understood. We hypothesize that Up_4A-induced aortic contraction is through COX-derived TXA_2 production, which requires activation of A_1 and/or A_3AR. Concentration responses to Up_4A were conducted in isolated aorta. The TXB_2 production, a metabolite of TXA_2, was also measured. Up_4A (10^{-9}-10^{-5} M) produced a concentration-dependent contraction >70%, which was markedly attenuated by COX and COX1 but not by COX2 inhibition. Notably, Up_4A-induced aortic contraction was blunted by both TX synthase inhibitor ozagrel and TXA_2 receptor (TP) antagonist SQ29548. Surprisingly, A_3AR deletion had no effect on Up_4A-induced contraction. Moreover, A_1AR deletion or antagonism as well as A_1/A_3AR deletion potentiated Up_4A-induced aortic contraction, suggesting a vasodilator influence of A_1AR. In contrast, non-selective purinergic P2 receptor antagonist PPADS significantly blunted Up_4A-induced aortic contraction to a similar extent as selective $P2X_1R$ antagonist MRS2159, the latter of which was further reduced by addition of ozagrel. Endothelial denudation almost fully attenuated Up_4A-induced contraction. Furthermore, Up_4A (3 μM) increased TXB_2 formation, which was inhibited by either MRS2159 or ozagrel. In conclusion, Up_4A-induced aortic contraction depends on activation of TX synthase and TP, which partially requires the activation of $P2X_1R$ but not A_1 or A_3AR through an endothelium-dependent mechanism.

1. Introduction

The endothelium releases a variety of vasodilators, such as nitric oxide (NO) and prostacyclin, and vasoconstrictors, such as endothelin and thromboxane (TX) A_2 [1,2]. A novel endothelium-derived vasoactive factor uridine adenosine tetraphosphate (Up_4A) has been initially identified as a vasoconstrictor in isolated kidney of rats [3]. Up_4A is the first dinucleotide found in living organisms that contains both purine and pyrimidine moieties and therefore can exert its vasoconstrictor effects through both the purinergic P1 (also known as ARs) and P2 receptors (P2Rs) [3,4]. Indeed, several in vitro studies have indicated that Up_4A induces vascular contraction through purinergic receptors. Thus, vasoconstriction was observed in rat renal artery through $P2X_1R$ [3], in rat aorta through P1 and P2XR [5], and in rat pulmonary arteries through P2YR [6]. Furthermore, vasoconstriction was also observed in mouse renal arterioles [7], mouse aorta [8] and rat mesenteric arteries [9], although Up_4A has been subsequently found to be able to exert a vasodilator effect in rat aorta [5], isolated perfused rat kidney [10] and porcine coronary arteries [11,12] as well as to induce hypotension in conscious rats [8]. The observations that plasma concentrations of Up_4A detected in juvenile hypertensive subjects are elevated [13], as well as intra-aortic injection of Up_4A increases mean arterial blood pressure in intact animal [3], suggest a role for Up_4A in the pathogenesis of hypertension. At post-receptor levels, Up_4A-induced vascular contraction was markedly attenuated by cyclooxygenase (COX) inhibition in

Abbreviations: AR, adenosine receptor; COX, cycloxygenase; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMSO, dimethyl sulphoxide; EDHF, endothelium-derived hyperpolarizing factor; KO, knockout; NS398, N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide; Ozagrel, (E)-3-[4-(imidazol-1-ylmethyl)phenyl]propenoic acid hydrochloride hydrate; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; SC560, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole; SQ29548, [1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; TXA_2, thromboxane; TP, thromboxane receptor; Up_4A, uridine adenosine tetraphosphate; U46619, 9,11-dideoxy-11α, 9α-epoxymethanoprostaglandin F2α.

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mouse aorta [8], implying a modulatory role of vasoconstrictor prostanoids in Up_4A-mediated vascular tone.

TXA_2, one of the vasoconstrictor prostanoids, is produced from arachidonic acid by COX pathway [1,14]. Subsequently, COX converts arachidonic acid into intermediate endoperoxide, the latter of which is catalyzed by TX synthase eventually leading to the production of TXA_2 [1]. By activating TXA_2 receptors (TP), TXA_2 has been reported to exert a vasoconstrictor effect in various vascular beds, thereby potentially contributing to the development of cardiovascular diseases such as hypertension [1]. Previous studies have shown that TXA_2 is produced and TP is activated by activation of P2XR and P2YR, which subsequently leads to vasoconstriction in canine basilar arteries [15], human umbilical and chorionic vessels [16] as well as rat aorta [17]. In addition to P2R, involvement of A_1AR has been observed in TXA_2-mediated vasoconstriction in feline pulmonary circulation [18]. Recently, we demonstrated that activation of A_3AR results in TXA_2 production, which leads to vascular contraction in mouse aorta [19]. All these observations indicate that TXA_2 can be generated via activation of purinergic receptors resulting in vascular contraction. More importantly, the observations that Up_4A activates TP resulting in renal vasoconstriction and Up_4A increases TXA_2 production in renal arteries of rats [20], suggest a link between Up_4A-mediated vascular contraction and the involvement of TXA_2. Although Up_4A-induced vasoconstriction in mouse aorta was attenuated by COX inhibition [8], the underlying mechanisms remain obscure.

Consequently, with particular focus on mouse aorta, we hypothesized that Up_4A-induced vascular contraction in mouse aorta is through COX-derived TXA_2 production, which requires activation of A_1AR and/or A_3AR. Specifically, we aimed to firstly investigate whether TXA_2 is produced and/or TP is activated in response to Up_4A that contributes to vascular contraction in mouse aorta, and secondly, to determine the A_1AR and/or A_3AR involvement in Up_4A-induced aortic contraction. Our findings from this study indicate that neither A_1AR nor A_3AR contributes to Up_4A-induced aortic contraction. Since activation of P2R, particularly $P2X_1R$, has been shown to be a vasoconstrictor purinergic receptor contributing to Up_4A-mediated vasoconstriction [3,21], the third aim of our study was to further explore the involvement of P2R, particularly $P2X_1R$, in Up_4A-induced vascular contraction in mouse aorta.

2. Materials and methods

2.1. Drugs and solutions

Acetylcholine, 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX), indomethacin, MRS2159, N-[2-(Cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS398), (E)-3-[4-(Imidazol-1-ylmethyl)phenyl]propenoic acid hydrochloride hydrate (ozagrel), phenylephrine, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) and 5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole (SC560) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Up_4A was obtained from Biolog Life Science (Bremen, Germany). [1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]-methyl]-7-xabicyclo[2.2.1] hept-2-yl]-5-heptenoic acid (SQ29-548) and thromboxane B_2 enzyme immunoassay kit (EIK) were purchased from Cayman Chemical (Ann Arbor, MI, USA). Indomethacin, DPCPX, NS398, SC560 and SQ29548 were firstly dissolved in DMSO. All subsequent dilutions (at least 1000 fold) and other drugs were obtained with distilled water. PPADS and MRS2159 were protected from light.

2.2. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee at School of Medicine, West Virginia University. Wild type (WT) mice (C57BL/6 background) were purchased from The Jackson Laboratory (Bar Harbor, ME). A_1AR knockout (KO), A_3AR KO and A_1/A_3AR double KO (DKO) mice were obtained from Dr. Stephen Tilley (University of North Carolina, Chapel Hill, NC). A_1AR and A_3AR KO mice, both backcrossed 12 generations to the WT, were bred to generate A_1/A_3AR double heterozygotes. Double heterozygotes were intercrossed, and 1/16th of the offspring were A_1/A_3AR DKO. A_1/A_3AR DKO breeding pairs were then established. Mice were caged in a 12:12-h light–dark cycles with free access to standard chow and water. Mice with age from 14 to 16 weeks of either sex were used in this study.

2.3. Tissue preparation and isometric force measurement

Mice were euthanized by anesthesia with pentobarbital sodium (65 mg/kg i.p.) followed by thoracotomy and removal of aorta. The aorta was cleaned by removing fat and connective tissues that was then cut transversely into 3 to 4 mm rings as described previously [19,22]. In a subset of rings, the endothelium was removed mechanically with a piece of thin wire by gently rolling it back and forward. Subsequently, the aortic rings were mounted vertically between two wire hooks and then suspended in 10 ml organ baths containing Krebs–Henseleit buffer. The Krebs–Henseleit buffer (pH 7.4) containing (in mM) 118 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_2, 25 $NaHCO_3$, 11 glucose and 2.5 $CaCl_2$ was maintained at 37 °C aerated with 95% O_2/5% CO_2. For measurement of isometric force response (with fixed range precision force transducer, TSD, 125 C; Biopac system), aortic rings were equilibrated for 60 min with a resting force of 1 g [22,23] and change of the Krebs solution at a 15 min interval. At the end of equilibration period, tissues were exposed to 50 mM KCl twice to check the contractility of individual aortic rings. Endothelial integrity was verified by observing relaxation to 1 μM acetylcholine after preconstriction with 1 μM phenylephrine [22]. The non-denuded rings that respond appropriately (relaxation >50%) and denuded rings that did not respond to acetylcholine were used in this study. Thereafter, rings were allowed to equilibrate in fresh organ bath fluid for 30 min before initiating different experimental protocols. Since previous study [8] as well as our current observation showed a very mild and transient contraction by Up_4A in aortic rings at basal condition (data not shown), all the experiments were performed upon a steady contraction by 1 μM phenylephrine. The aortic rings that did not elicit reproducible and stable contraction with phenylephrine were excluded from the study. In experiments where the effect of an antagonist was measured, it was added 30 min before preconstriction with phenylephrine and was present throughout the experiments. Only one protocol was executed per ring and within one protocol all rings were obtained from different animals.

2.4. Organ bath experimental protocols

Preconstricted aortic rings were subjected to Up_4A concentration responses (10^{-9}–10^{-5} M). In accordance with previous studies [8], Up_4A, at a dose of 10^{-5} M, produced vasoconstriction followed by vasodilation in mouse aorta, indicating a biphasic effect of Up_4A. Since the aim of the present study was to elucidate the mechanisms of Up_4A-induced contraction in mouse aorta, the observation that Up_4A-induced vasodilation at a dose of 10^{-5} M was excluded.

Up_4A-induced aortic contraction was observed to be attenuated by COX inhibition [8], to investigate which COX subtype was involved in this process, rings were subjected to Up_4A (10^{-9}–10^{-5} M) in the absence and presence of non-selective COX inhibitor indomethacin (10 μM) [19], selective COX1 inhibitor SC560 (10 nM) [19] and selective COX2 inhibitor NS398 (1 μM) [19]. To test whether the involvement of vasoconstrictor prostanoids was through TXA_2 production or TP activation, Up_4A concentration responses were performed in aortic rings in the absence and presence of TX synthase inhibitor ozagrel (10 μM) [24] and TP antagonist SQ29548 (1 μM) [19], respectively.

Since both A_1AR and A_3AR have been shown to be involved in TXA_2-mediated vascular contraction [18,19], aortic rings from WT, A_1AR KO, A_3AR KO and A_1/A_3AR DKO mice were exposed to Up_4A concentration