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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 (COX). We and others demonstrated that activation of A_1 or A_3 adenosine receptors (AR) 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_3 AR through an endothelium-dependent mechanism.

Graphical Abstract

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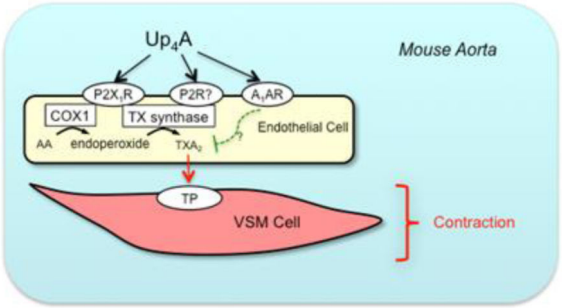

Keywords

Up_4A; mouse aorta; $P2X_1$; thromboxane; endothelium; vasoconstriction

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 (P2R) (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 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_4Amediated 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 (SQ29548) and thromboxane B2 enzyme immunoassay (EIK) kit 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 $1/16^{th}$ 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 wk 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 dilation 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 responses (10^{-9}–10^{-5} M). In addition, aortic rings of WT were subjected to Up_4A in the absence and presence of selective A_1AR antagonist DPCPX (10 nM) (25). To investigate the involvement of P2R in Up_4A-induced vascular contraction, aortic rings of WT were exposed to Up_4A in the absence and presence of non-selective P2R antagonist PPADS (10 μM) (12) and selective $P2X_1R$ antagonist MRS2159 (30 μM) (26), respectively.

To assess whether TXA_2 production requires activation of $P2X_1R$, aortic rings were treated with Up_4A in the absence and presence of MRS2159, ozagrel and the combination of MRS2159 and ozagrel.

To investigate the possible endothelial involvement of Up_4A-induced aortic contraction, Up_4A concentration responses were conducted in endothelium –non-denuded and –denuded rings. To identify the compartment in which $P2X_1R$ locates to generate TXA_2 in response to Up_4A, denuded aortic rings were exposed to Up_4A in the absence and presence of MRS2179 and ozagrel, respectively. The various antagonists used in the present study do not interfere with 1 μM phenylephrine response within each figure panels and there is no significant difference in 1 μM phenylephrine response between WT and KO mice (data not shown).

2.5 Measurement of TXA_2 release

TXB_2, a metabolite of TXA_2, was measured according to protocols from previous studies (20, 27), with slight modification. Briefly, cleaned mouse aortic rings were incubated at 37°C for 60 min in a siliconized tube containing 1 ml of Krebs-Henseleit buffer, as described for organ bath studies. After incubation, rings were treated with MRS2159 (30 μM) or ozagrel (10 μM), and incubated for another 30 min in 500 μl Krebs buffer. Thereafter, Up_4A (3 μM) or vehicle (water) was added for 10 min. Then the aortic rings were removed and the tubes were freeze-clamped in liquid nitrogen and stored at −80°C for subsequent analysis. TXB_2 was measured using an EIA kit (Cayman Chemicals, Ann Arbor, MI, USA). The undiluted 50 μl samples were used. The various assays were performed as described in the manufacturer's booklet. The amount of TXB_2 is expressed in picograms per milligram wet weight of the aortic rings.

2.6 Data analysis and statistics

Vascular contraction responses to Up_4A were expressed as percentage of contraction to phenylephrine. The effects of drug treatment on the Up_4A concentration responses were assessed using two-way ANOVA for repeated measures. The effects of drug treatment on Up_4A-induced TXA_2 production were analyzed using one-way ANOVA. Statistical significance was accepted when $P < 0.05$ (two-tailed). Data are presented as means ± SEM.

3. Results

3.1 Involvement of COX1 in Up_4A-induced aortic contraction

In preconstricted aortic rings, Up_4A produced a concentration-dependent contraction over 70% (Fig. 1). In accordance with previous studies (8), Up_4A-induced aortic contraction was markedly attenuated by non-selective COX inhibitor indomethacin (Fig. 1A). Furthermore, Up_4A-induced aortic contraction was significantly attenuated by selective COX1 inhibitor SC560 (Fig. 1B), but not by selective COX2 inhibitor NS398 (Fig. 1C). These observations indicate that Up_4A-induced contraction in mouse aorta is attributed to vasoconstrictor prostanoids produced by COX1 activation.

3.2 Involvement of TXA_2 in Up_4A-induced aortic contraction

To investigate whether the involvement of vasoconstrictor prostanoids in Up_4A-induced aortic contraction is TXA_2, we performed Up_4A concentration responses in isolated aortic rings in the absence and presence of either TX synthase inhibition or TP antagonism. Up_4A-induced aortic contraction was dramatically attenuated by TX synthase inhibitor ozagrel (Fig. 2A) and TP antagonist SQ29548 (Fig. 2B), respectively. These findings indicate that the involvement of vasoconstrictor prostanoids in Up_4A-induced aortic contraction appears to be through TX synthase and TP activation.

3.3 Involvement of purinergic P1 receptors in Up_4A-induced aortic contraction

Since both A_1AR and A_3AR have been shown to be involved in TXA_2-mediated vascular contraction (18, 19), we next studied the involvements of A_1AR and A_3AR in Up_4A-induced aortic contraction. Up_4A induced a concentration-dependent aortic contraction in WT mice, which was comparable to that in A_3AR KO mice (Fig. 3A). Surprisingly, Up_4A-induced aortic contraction was potentiated in A_1AR KO as compared to WT mice (Fig. 3B). A similar observation was also found in A_1/A_3AR DKO mice in which Up_4A-induced aortic contraction was enhanced as compared to WT mice (Fig. 3C). These findings suggest that A_3ARs does not appear to be involved in Up_4A-induced contraction in mouse aorta. However, A_1AR may exert a vasodilator rather than vasoconstrictor influence in Up_4A-induced aortic contraction.

The observation that Up_4A-induced aortic contraction was enhanced in A_1AR KO or A_1/A_3AR DKO mice may be due to a compensatory mechanism that often occurs using genetically modified mice (28). To further confirm the role of A_1AR in Up_4A-induced aortic contraction, we performed concentration responses to Up_4A in isolated aortic rings from WT mice in the absence and presence of pharmacological inhibition by the selective A_1AR antagonist. Similar to the observations from A_1AR KO or A_1/A_3AR DKO, selective A_1AR antagonist DPCPX significantly potentiated Up_4A-induced contraction in mouse aorta (Fig. 3D). This finding supports the observation that A_1AR may exert a vasodilator influence, which suppresses Up_4A-induced vascular contraction in mouse aorta.

3.4 Involvement of purinergic P2 receptors in Up_4A-induced aortic contraction

Based on the findings that neither A_1AR nor A_3AR exerts a vasoconstrictor influence contributing to Up_4A-induced aortic contraction, we next investigated the involvement of

P2R in this process. Non-selective P2R antagonist PPADS (Fig. 4A) and selective $P2X_1R$ antagonist MRS2159 (Fig. 4B) significantly attenuated Up_4A-induced vascular contraction in mouse aorta, respectively. To further study whether Up_4A-induced aortic contraction via TXA_2 production requires activation of $P2X_1R$, isolated aortic rings were exposed to MRS2159, ozagrel or the combination of both MRS2159 and ozagrel. Up_4A-induced aortic contraction was significantly reduced by MRS2159, which was slightly but significantly further attenuated by addition of ozagrel (Fig. 5). However, the inhibition of Up_4A-induced aortic contraction by a combination of MRS2159 and ozagrel was similar in magnitude to that obtained by ozagrel alone (Fig. 5). All together, these observations indicate that Up_4A-induced vascular contraction in mouse aorta is through TXA_2 production, which partially requires the activation of $P2X_1R$.

3.5 Endothelium-dependency of aortic contraction produced by Up_4A

To elucidate the role of endothelium in Up_4A-induced aortic contraction, endothelium-denuded aortic rings were subjected to Up_4A concentration responses in the absence and presence of $P2X_1R$ antagonist MRS2159 and TX synthase inhibitor ozagrel. Up_4A-induced aortic contraction was almost completely blunted by endothelium denudation either with or without MRS2159 or ozagrel (Fig. 6), indicating that Up_4A-induced contraction in mouse aorta is mainly endothelium-dependent.

3.6 TXA_2 production generated by Up_4A in isolated aortic rings

To further test our finding that TXA_2 is involved in Up_4A-induced aortic contraction, TXB_2, a metabolite of TXA_2, was measured in isolated intact aortic rings upon Up_4A stimulation. The level of TXB_2 was greatly increased in Up_4A (3 μM)-treated tissues as compared to vehicle (Fig. 7). Furthermore, Up_4A-increased TXB_2 level was dramatically inhibited by either $P2X_1R$ antagonist MRS2159 or TX synthase inhibitor ozagrel (Fig. 7). These findings suggest that Up_4A is capable of generating TXA_2, which partially requires activation of $P2X_1R$ in mouse aorta.

4. Discussion

The main findings of the present study are: 1) Up_4A produced a concentration-dependent vascular contraction (>70%), which was attenuated by non-selective COX and selective COX1 but not COX2 inhibition; 2) Up_4A-induced aortic contraction was markedly blocked by TX synthase inhibition and TP antagonism, respectively; 3) A_3AR deletion had no effect on Up_4A-induced aortic contraction, whereas A_1AR deletion or antagonism as well as A_1/A_3AR deletion potentiated Up_4A-induced contraction; 4) In contrast, non-selective P2R antagonist PPADS attenuated Up_4A-induced contraction, while selective $P2X_1R$ antagonist MRS2159 similarly attenuated Up_4A-induced aortic contraction, which was further reduced by TX synthase inhibitor ozagrel; 5) Furthermore, endothelial denudation almost fully blunted Up_4A-induced aortic contraction either with or without MRS2159 or ozagrel; 6) and finally, TXA_2 production was significantly increased by Up_4A in intact isolated aortic rings, which was inhibited by either MRS2159 or ozagrel. The implications of these findings are discussed below.

Up_4A was first identified as a potent endothelium-derived vasoconstrictor in rat perfused kidney (3). Several subsequent studies have confirmed that Up_4A produces vasoconstriction in rat renal artery (3), aorta (5), gastric smooth muscle (29) and pulmonary arteries (6). Furthermore, vasoconstriction was observed in mouse renal arterioles (7) and aorta (8). Although, there is evidence that Up_4A can produce vasodilation in isolated aortic rings of rats (5), porcine coronary small arteries (11, 12), human and mouse colon (30) as well as induce hypotension in conscious rats (8). All together, these studies suggest that the vascular responses to Up_4A likely depend on the type of vascular beds and species studied (4).

In mouse aorta, Hansen and colleagues (8) demonstrated that in preconstricted aortic rings, cumulative addition (10^{-7}–10^{-5} M) of Up_4A produced vasoconstriction followed by relaxation at a dose of 10^{-5} M, indicating a biphasic effect of Up_4A. Consistent with the vasodilator effect induced by Up_4A at a dose of 10^{-5} M, we observed a similar relaxation of 41 ± 0.06% in the present study as compared to the report by Hansen et. al. (46 ± 6% relaxation) (8). However, cumulative addition of Up_4A (10^{-9}–10^{-5} M) produced a concentration-dependent contraction over 70%, which was inconsistent with the previous study in which cumulative addition of Up_4A (10^{-7}–10^{-5} M) caused aortic contraction of 18 ± 2%, 76 ± 16% and 18 ± 6%, respectively (8). This disparity may not be attributed to the different strain of animals (the same C57BL/6 mice with similar age range), but might due to the different number of animals (n) used in the studies in which variable vascular responses to Up_4A between each individual animals may occur, as a large number of control animals were pooled in the present study (n=49) as opposed to the study by Hansen et. al. with an ‘n’ of only six (8). For the sake of comparing vasoconstrictor effect of Up_4A (>70%), another vasoconstrictor agonist U46619 (TXA_2 analogue 9,11-Dideoxy-11α, 9α - epoxymethanoprostaglandin F2α) concentration responses (10^{-9} M–10^{-6} M) were performed in the same preparation that produced a dose-dependent contraction up to ~240%, which almost reaches the maximal contraction. Together with the vasoconstrictor effects of ATP (~50%) (31) and activation of ARs (~30%) in isolated aorta, this may indicate that Up_4A is a relatively potent vasoconstrictor, which submaximally contracts mouse aorta. Notably, infusion of Up_4A (10^{-9}–10^{-5} M) into isolated mouse hearts results in a dose-dependent reduction in coronary flow by ~40% (unpublished) as well as intra-aortic injection of Up_4A (100 nmol) increases mean arterial blood pressure by ~25% in anesthetized rats (3), suggesting a potential vasoconstrictor influence of Up_4A *in vivo*.

In accordance with previous finding by Hansen et. al. (8), Up_4A-induced aortic contraction was markedly attenuated by non-selective COX inhibition with indomethacin, suggesting an involvement of vasoconstrictor prostanoids. Subsequent observations that Up_4A-induced aortic contraction was blunted by selective-COX1 but not selective COX2 inhibition further supports the concept that activation of COX1 but not COX2 primarily contributes to generation of vasoconstrictor prostanoids in mouse aorta (32, 33). On the other hand, the lack of involvement of COX2 in Up_4A-mediated aortic contraction could be due to the fact that COX2, an inducible enzyme, predominantly plays a role under inflammatory /diseased conditions (34). Indeed, one of the recent studies in diabetes showed that COX2 but not COX1 contributes to Up_4A-increased renal contraction (20). Future studies on various disease models are needed to further explore our understanding of these mechanisms. As

reported earlier that TXA_2 production is abundant in mouse aorta at basal conditions (32) and Up_4A is capable of producing TXA_2 in renal arteries of rats (20), Up_4A may also potentially increase TXA_2 in mouse aorta causing vasoconstriction. Indeed, Up_4A-induced aortic contraction was significantly reduced by both TP antagonism and TX synthase inhibition, suggesting that Up_4A-induced contraction in mouse aorta is not only attributed to TP activation but also to TXA_2 production. These findings are further supported by the observation that Up_4A-increased TXA_2 level in intact isolated mouse aorta was largely decreased by TXA_2 sysnthase inhibition. Together with the involvement of COX1, these findings support our hypothesis that TXA_2, likely derived from COX1 activation, contributes to Up_4A-mediated aortic contraction in mouse aorta.

As a novel extracellular dinucleotide, the vascular effects of Up_4A observed in previous studies have been challenged to be through its degradation to ATP or adenosine (3, 5, 11). Although both ATP and activation of ARs have been shown to produce aortic contraction through generation of TXA_2 (19, 31), ATP (31) and activation of ARs (19) affected vascular tone much less (~50% and ~30%, respectively) than Up_4A (>70%), indicating that these degradation products can only account for a small part, if any, of the Up_4A effects, as proposed that Up_4A might also be a stimulator of endothelial ATP release (3).

Like other nucleotides, Up_4A exerts its vascular influence through activation of purinergic receptors (3, 4). Thus, Up_4A induces vasoconstriction in rat perfused kidney through $P2X_1R$ and $P2Y_1R$ (10), in rat aorta through P1R and P2XR (5), and in rat pulmonary arteries through P2YR (6), while Up_4A produces vasodilation in rat perfused kidney through $P2Y_1R$ and $P2Y_2R$ (10), in porcine coronary arteries through $A_{2A}AR$, $P2X_1R$ and $P2Y_1R$ (12) and in human and mouse colon through $P2Y_1R$ (30). Surprisingly, Up_4A-mediated vascular contraction in mouse aorta in the present study was not affected by A_3AR deletion, although one of our previous observations showed that activation of A_3AR contributes to mouse aortic contraction (19). Moreover, Up_4A-induced aortic contraction was significantly potentiated by either A_1AR or A_1/A_3AR deletion, which was further supported by an enhancing effect with pharmacological inhibition of A_1AR (DPCPX). The enhancement with DPCPX in Up_4A-induced aortic contraction in WT was greater than that in A_1AR KO or A_1/A_3AR DKO mice. This difference in enhancement between pharmacological agent in WT and genetically modified mice is likely due to the upregulation of other purinergic receptors in A_1AR KO or A_1/A_3AR DKO that compensates for the loss of A_1AR (28), as we previously showed that there is an upregulation of $A_{2B}AR$ in A_1AR KO mice (28). The vasodilator $A_{2B}AR$ is likely activated by Up_4A to counteract the unveiled vasoconstrictor effect due to the loss of A_1AR, thereby leading to a less enhancement of Up_4A effect in A_1AR KO or A_1/A_3AR DKO as compared to that with DPCPX in WT. Taken together, these findings suggest that A_3AR does not appear to be involved in Up_4A-induced aortic contraction, and activation of A_1AR may exert a vasodilator influence in this process. In contrast to the generally accepted role of A_1AR exerting a vasoconstrictor influence in the cardiovascular system (28, 35, 36), several previous studies have shown that selective A_1AR agonist induces vasodilation in porcine coronary arteries (37), adenosine-mediated activation of A_1AR leads to vasodilation in rat diaphragmatic arterioles (38) and activation of A_1AR by adenosine contributes to hypoxia-induced vasodilation in perfused rabbit hearts (39). Moreover, at cellular levels, activation of A_1AR has been shown to mediate release of

NO from the endothelium of rat aorta (40). However, we could not exclude the involvement of vasodilator influence of A_1AR in Up_4A-induced aortic contraction is through other indirect mechanisms, as the interaction between A_1AR and $P2Y_1$R exists (41). Activation of $P2Y_1$R has been shown to exert a vasodilator influence in vasculature. Thus, Up_4A activates $P2Y_1$R to produce vasodilation in rat perfused kidney (10), porcine coronary arteries (12), as well as human and mouse colons (30). Future studies addressing the role of A_1AR in this process will bring greater insights into the purinergic receptor-regulated vascular tone.

In contrast to the observations that neither A_1AR nor A_3AR exerts a vasoconstrictor influence contributing to Up_4A-induced aortic contraction, activation of $P2X_1$R, at least in part, accounts for Up_4A-induced vascular contraction in mouse aorta, as evidenced by a significant attenuation of Up_4A-induced aortic contraction by both non-selective P2R antagonist PPADS as well as selective $P2X_1$R antagonist MRS2159. The involvement of $P2X_1$R in this process is in agreement with previous studies that activation of $P2X_1$R contributes to both Up_4A-mediated contraction in renal arteries (3) and NADPH-mediated contraction in aorta (42). Furthermore, there is some evidence linking TXA_2 production or TP activation to activation of P2R. Thus, P2XR-agonist-induced vasoconstriction was attenuated by TXA_2 antagonist in canine basilar arteries through endothelium-dependent mechanism (15), activation of both $P2Y_1$R and $P2Y_2$R produced vasoconstriction in human umbilical and chorionic vessels, which was blunted by TP antagonist (16), and ATP-induced contraction in rat aorta was attenuated by TP antagonist (31). Consistent with these observations, in the present study, Up_4A increased TXA_2 production in isolated aorta was inhibited by $P2X_1$R antagonist MRS2159. Of note, Up_4A-induced aortic contraction was significantly attenuated by MRS2159, which was further blunted by addition of TX synthase inhibitor ozagrel. Our findings suggest that Up_4A-induced aortic contraction mediated by TXA_2 is, in part, through activation of $P2X_1$R. The reduction of Up_4A-induced aortic contraction by $P2X_1$R antagonist MRS2159 was similar in magnitude to that obtained by non-selective P2R antagonist PPADS, which could not exclude the possibility of involvement of additional P2R subtypes (both P2XR and P2YR) other than $P2X_1$R in Up_4A-mediated aortic contraction. Since PPADS blocks most, but not all, of P2R subtypes, and PPADS does not inhibit $P2X_1$R, as evidenced by precious studies that PPADS blocked $P2X_2$R, $P2X_3$R, $P2X_5$R (43), $P2X_7$R (44), $P2Y_1$R (45), $P2Y_2$R, $P2Y_4$R (46) and $P2Y_6$R (47). Notably, $P2X_1$R-mediated renal vasoconstriction in response to Up_4A was not blocked by PPADS but blocked by another non-selective P_2R antagonist suramin (10). The limitation for the relation between TXA_2 generation and activation of $P2X_1$R is that whether the generation of TXA_2 in response to Up_4A was truly of endothelial origin or from the transcellular metabolism of endoperoxide is unknown, as various prostaglandins contract the smooth muscle cells by activating TP (31). On the other hand, methodological intricacies are compounded by lack of selective $P2X_1$R agonist to explore in detail the coupling of TXA_2 production to activation of $P2X_1$R (16). Future studies regarding this issue will confirm the coupling between TXA_2 production and $P2X_1$R activation.

P2R in the vasculature are present on endothelial cells as well as vascular smooth muscle cells (VSMC). Activation of P2R on endothelial cells is generally accepted to produce vasodilation, whereas activation of P2R on VSMC leads to vasoconstriction (48). Indeed, previous studies have shown that activation of endothelial $P2X_1$R in response to Up_4A

results in vasodilation in porcine coronary arteries (12), whereas activation of $P2X_1R$ by Up_4A on VSMC produces vasoconstriction in rat perfused kidney (3, 4). In contrast to this concept, the observation that endothelium-denudation almost fully attenuated Up_4A-induced aortic contraction both in the absence and presence of $P2X_1R$ or TXA_2 inhibition indicates that Up_4A-induced contraction is mainly endothelium-dependent, and Up_4A generates TXA_2 production requiring activation of $P2X_1R$ on endothelial cells. Similar phenomenon has been observed in rat aorta in which activation of endothelial P2XR by ATP leads to endothelial TXA_2 production (31), and activation of $P2X_1R$ located on endothelial cells has been shown to contribute to NADPH-mediated vascular contraction in mouse aorta (42).

5. Conclusions

The present study demonstrates that Up_4A-induced vascular contraction in mouse aorta is attributed to TXA_2 production, which partially requires activation of $P2X_1R$ through an endothelium-dependent mechanism. As plasma levels of Up_4A in juvenile hypertensive subjects are significantly elevated (13) and intra-aortic injection of Up_4A increases mean arterial blood pressure in intact animal (3), suggesting a role for Up_4A in pathogenesis of hypertension, further studies investigating the altered vascular responses to Up_4A in hypertensive conditions and the contribution of TXA_2 to Up_4A-mediated changes in blood pressure will provide novel therapeutic targets in this process.

Acknowledgments

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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),3α4α]]-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

References

1. Feletou M, Huang Y, Vanhoutte PM. Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. Br J Pharmacol. 2011; 164:894–912. [PubMed: 21323907]
2. Laughlin MH, Davis MJ, Secher NH, van Lieshout JJ, Arce-Esquivel AA, Simmons GH, Bender SB, Padilla J, Bache RJ, Merkus D, Duncker DJ. Peripheral circulation. Compr Physiol. 2012; 2:321–447. [PubMed: 23728977]
3. Jankowski V, Tolle M, Vanholder R, Schonfelder G, van der Giet M, Henning L, Schluter H, Paul M, Zidek W, Jankowski J. Uridine adenosine tetraphosphate: a novel endothelium- derived vasoconstrictive factor. Nat Med. 2005; 11:223–227. [PubMed: 15665829]
4. Matsumoto T, Tostes RC, Webb RC. The role of uridine adenosine tetraphosphate in the vascular system. Adv Pharmacol Sci. 2011; 2011:435132. [PubMed: 22110488]
5. Linder AE, Tumbri M, Linder FF, Webb RC, Leite R. Uridine adenosine tetraphosphate induces contraction and relaxation in rat aorta. Vascul Pharmacol. 2008; 48:202–207. [PubMed: 18467183]
6. Gui Y, Walsh MP, Jankowski V, Jankowski J, Zheng XL. Up4A stimulates endothelium-independent contraction of isolated rat pulmonary artery. Am J Physiol Lung Cell Mol Physiol. 2008; 294:L733–L738. [PubMed: 18192588]
7. Jankowski V, Patzak A, Herget-Rosenthal S, Tran TN, Lai EY, Gunthner T, Buschmann I, Zidek W, Jankowski J. Uridine adenosine tetraphosphate acts as an autocrine hormone affecting glomerular filtration rate. J Mol Med (Berl). 2008; 86:333–340. [PubMed: 18250997]
8. Hansen PB, Hristovska A, Wolff H, Vanhoutte P, Jensen BL, Bie P. Uridine adenosine tetraphosphate affects contractility of mouse aorta and decreases blood pressure in conscious rats and mice. Acta Physiol (Oxf). 2010; 200:171–179. [PubMed: 20384597]
9. Matsumoto T, Tostes RC, Webb RC. Alterations in vasoconstrictor responses to the endothelium-derived contracting factor uridine adenosine tetraphosphate are region specific in DOCA-salt hypertensive rats. Pharmacol Res. 2012; 65:81–90. [PubMed: 21933714]
10. Tolle M, Schuchardt M, Wiedon A, Huang T, Klockel L, Jankowski J, Jankowski V, Zidek W, van der Giet M. Differential effects of uridine adenosine tetraphosphate on purinoceptors in the rat isolated perfused kidney. Br J Pharmacol. 2010; 161:530–540. [PubMed: 20880394]
11. Zhou Z, Merkus D, Cheng C, Duckers HJ, Jan Danser AH, Duncker DJ. Uridine adenosine tetraphosphate is a novel vasodilator in the coronary microcirculation which acts through purinergic P1 but not P2 receptors. Pharmacol Res. 2013; 67:10–17. [PubMed: 23063485]
12. Zhou Z, de Wijs-Meijler D, Lankhuizen I, Jankowski J, Jankowski V, Jan Danser AH, Duncker DJ, Merkus D. Blunted coronary vasodilator response to uridine adenosine tetraphosphate in post-infarct remodeled myocardium is due to reduced P1 receptor activation. Pharmacol Res. 2013; 77:22–29. [PubMed: 23994209]
13. Jankowski V, Meyer AA, Schlattmann P, Gui Y, Zheng XL, Stamcou I, Radtke K, Tran TN, van der Giet M, Tolle M, Zidek W, Jankowski J. Increased uridine adenosine tetraphosphate concentrations in plasma of juvenile hypertensives. Arterioscler Thromb Vasc Biol. 2007; 27:1776–1781. [PubMed: 17569882]
14. Tangand EH, Vanhoutte PM. Prostanoids and reactive oxygen species: team players in endothelium-dependent contractions. Pharmacol Ther. 2009; 122:140–149. [PubMed: 19285526]
15. Shirahase H, Usui H, Shimaji H, Kurahashi K, Fujiwara M. Endothelium-independent and endothelium-dependent contractions mediated by P2X- and P2Y-purinoceptors in canine basilar arteries. J Pharmacol Exp Ther. 1991; 256:683–688. [PubMed: 1994001]
16. Buvinic S, Poblete MI, Donoso MV, Delpiano AM, Briones R, Miranda R, Huidobro-Toro JP. P2Y1 and P2Y2 receptor distribution varies along the human placental vascular tree: role of nucleotides in vascular tone regulation. J Physiol. 2006; 573:427–443. [PubMed: 16543271]

17. Mombouliand JV, Vanhoutte PM. Purinergic endothelium-dependent and -independent contractions in rat aorta. Hypertension. 1993; 22:577–583. [PubMed: 8406663]
18. Neely CF, Haile DM, Cahill BE, Kadowitz PJ. Adenosine and ATP produce vasoconstriction in the feline pulmonary vascular bed by different mechanisms. J Pharmacol Exp Ther. 1991; 258:753–761. [PubMed: 1832463]
19. Ansari HR, Nadeem A, Tilley SL, Mustafa SJ. Involvement of COX-1 in A3 adenosine receptor-mediated contraction through endothelium in mice aorta. Am J Physiol Heart Circ Physiol. 2007; 293:H3448–H3455. [PubMed: 17921329]
20. Matsumoto T, Watanabe S, Kawamura R, Taguchi K, Kobayashi T. Enhanced uridine adenosine tetraphosphate-induced contraction in renal artery from type 2 diabetic Goto-Kakizaki rats due to activated cyclooxygenase/thromboxane receptor axis. Pflugers Arch. 2014; 466:331–342. [PubMed: 23900807]
21. Gui Y, Wang Z, Sun X, Walsh MP, Li JJ, Gao J, Zheng XL. Uridine adenosine tetraphosphate induces contraction of airway smooth muscle. Am J Physiol Lung Cell Mol Physiol. 2011; 301:L789–L794. [PubMed: 21840961]
22. Pradhan I, Zeldin DC, Ledent C, Mustafa JS, Falck JR, Nayeem MA. High salt diet exacerbates vascular contraction in the absence of adenosine A(2)A receptor. J Cardiovasc Pharmacol. 2014; 63:385–394. [PubMed: 24390173]
23. Ponnoth DS, Nayeem MA, Kunduri SS, Tilley SL, Zeldin DC, Ledent C, Mustafa SJ. Role of omega-hydroxylase in adenosine-mediated aortic response through MAP kinase using A2A-receptor knockout mice. Am J Physiol Regul Integr Comp Physiol. 2012; 302:R400–R408. [PubMed: 22160543]
24. Miike T, Kunishiro K, Kanda M, Azukizawa S, Kurahashi K, Shirahase H. Impairment of endothelium-dependent ACh-induced relaxation in aorta of diabetic db/db mice--possible dysfunction of receptor and/or receptor-G protein coupling. Naunyn Schmiedebergs Arch Pharmacol. 2008; 377:401–410. [PubMed: 18228001]
25. Headrick JP, Gauthier NS, Morrison RR, Matherne GP. Chronotropic and vasodilatory responses to adenosine and isoproterenol in mouse heart: effects of adenosine A1 receptor overexpression. Clin Exp Pharmacol Physiol. 2000; 27:185–190. [PubMed: 10744345]
26. Wee S, Peart JN, Headrick JP. P2 purinoceptor-mediated cardioprotection in ischemic-reperfused mouse heart. J Pharmacol Exp Ther. 2007; 323:861–867. [PubMed: 17855479]
27. Gluais P, Paysant J, Badier-Commander C, Verbeuren T, Vanhoutte PM, Feletou M. In SHR aorta, calcium ionophore A-23187 releases prostacyclin and thromboxane A2 as endothelium-derived contracting factors. Am J Physiol Heart Circ Physiol. 2006; 291:H2255–H2264. [PubMed: 16798820]
28. Teng B, Fil D, Tilley SL, Ledent C, Krahn T, Mustafa SJ. Functional and RNA expression profile of adenosine receptor subtypes in mouse mesenteric arteries. J Cardiovasc Pharmacol. 2013; 61:70–76. [PubMed: 23288107]
29. Yuan W, Wang Z, Li J, Li D, Liu D, Bai G, Walsh MP, Gui Y, Zheng XL. Uridine adenosine tetraphosphate induces contraction of circular and longitudinal gastric smooth muscle by distinct signaling pathways. IUBMB Life. 2013; 65:623–632. [PubMed: 23671036]
30. Durnin L, Hwang SJ, Kurahashi M, Drumm BT, Ward SM, Sasse KC, Sanders KM, Mutafova-Yambolieva VN. Uridine adenosine tetraphosphate is a novel neurogenic P2Y1 receptor activator in the gut. Proc Natl Acad Sci U S A. 2014; 111:15821–15826. [PubMed: 25341729]
31. Gluais P, Vanhoutte PM, Feletou M. Mechanisms underlying ATP-induced endothelium-dependent contractions in the SHR aorta. Eur J Pharmacol. 2007; 556:107–114. [PubMed: 17126320]
32. Qi Z, Cai H, Morrow JD, Breyer MD. Differentiation of cyclooxygenase 1- and 2-derived prostanoids in mouse kidney and aorta. Hypertension. 2006; 48:323–328. [PubMed: 16801485]
33. Zhou Y, Luo W, Zhang Y, Li H, Huang D, Liu B. Cyclo-oxygenase-1 or -2-mediated metabolism of arachidonic acid in endothelium-dependent contraction of mouse arteries. Exp Physiol. 2013; 98:1225–1234. [PubMed: 23538462]
34. Iezzi A, Ferri C, Mezzetti A, Cipollone F. COX-2: friend or foe? Curr Pharm Des. 2007; 13:1715–1721. [PubMed: 17584101]

35. Tawfik HE, Teng B, Morrison RR, Schnermann J, Mustafa SJ. Role of A1 adenosine receptor in the regulation of coronary flow. Am J Physiol Heart Circ Physiol. 2006; 291:H467–H472. [PubMed: 16517942]

36. Wang Y, Yang JN, Arner A, Boels PJ, Fredholm BB. Adenosine A(1) receptors and vascular reactivity. Acta Physiol (Oxf). 2010; 199:211–220. [PubMed: 20132147]

37. Merkel LA, Lappe RW, Rivera LM, Cox BF, Perrone MH. Demonstration of vasorelaxant activity with an A1-selective adenosine agonist in porcine coronary artery: involvement of potassium channels. J Pharmacol Exp Ther. 1992; 260:437–443. [PubMed: 1310735]

38. Danialou G, Vicaut E, Sambe A, Aubier M, Boczkowski J. Predominant role of A1 adenosine receptors in mediating adenosine induced vasodilatation of rat diaphragmatic arterioles: involvement of nitric oxide and the ATP-dependent K+ channels. Br J Pharmacol. 1997; 121:1355–1363. [PubMed: 9257914]

39. Nakhostineand N, Lamontagne D. Adenosine contributes to hypoxia-induced vasodilation through ATP-sensitive K+ channel activation. Am J Physiol. 1993; 265:H1289–H1293. [PubMed: 8238416]

40. Rayand CJ, Marshall JM. The cellular mechanisms by which adenosine evokes release of nitric oxide from rat aortic endothelium. J Physiol. 2006; 570:85–96. [PubMed: 16239264]

41. Yoshioka K, Saitoh O, Nakata H. Heteromeric association creates a P2Y-like adenosine receptor. Proc Natl Acad Sci U S A. 2001; 98:7617–7622. [PubMed: 11390975]

42. Judkins CP, Sobey CG, Dang TT, Miller AA, Dusting GJ, Drummond GR. NADPH-induced contractions of mouse aorta do not involve NADPH oxidase: a role for P2X receptors. J Pharmacol Exp Ther. 2006; 317:644–650. [PubMed: 16407465]

43. Burnstock G. Purine and pyrimidine receptors. Cell Mol Life Sci. 2007; 64:1471–1483. [PubMed: 17375261]

44. Mezzaroma E, Toldo S, Farkas D, Seropian IM, Van Tassell BW, Salloum FN, Kannan HR, Menna AC, Voelkel NF, Abbate A. The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. Proc Natl Acad Sci U S A. 2011; 108:19725–19730. [PubMed: 22106299]

45. Ju YK, Huang W, Jiang L, Barden JA, Allen DG. ATP modulates intracellular Ca2+ and firing rate through a P2Y1 purinoceptor in cane toad pacemaker cells. J Physiol. 2003; 552:777–787. [PubMed: 12949218]

46. Rayment SJ, Latif ML, Ralevic V, Alexander SP. Evidence for the expression of multiple uracil nucleotide-stimulated P2 receptors coupled to smooth muscle contraction in porcine isolated arteries. Br J Pharmacol. 2007; 150:604–612. [PubMed: 17262017]

47. Schreiberand R, Kunzelmann K. Purinergic P2Y6 receptors induce Ca2+ and CFTR dependent Cl- secretion in mouse trachea. Cell Physiol Biochem. 2005; 16:99–108. [PubMed: 16121038]

48. Burnstock G. Control of vascular tone by purines and pyrimidines. Br J Pharmacol. 2010; 161:527–529. [PubMed: 20880393]

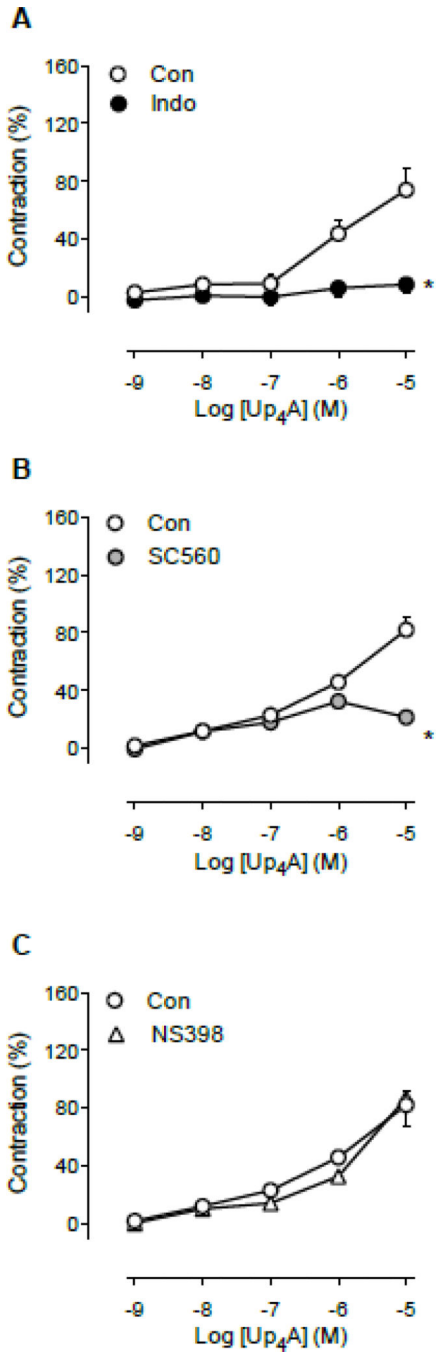

Figure 1.

Effects of non-selective cyclooxygenase (COX), selective COX1 and COX2 inhibition on Up_4A-induced vasoconstriction. Shown are the concentration-responses to Up_4A in mouse aorta from wild-type (WT) mice in the absence and presence of 10 μM of the non-selective COX inhibitor indomethacin (Indo) (n=13 for both Con and Indo; panel A), 10 nM of the selective COX1 inhibitor SC560 (n=11 for Con; n=12 for SC560; panel B), and 1 μM of the selective COX2 inhibitor NS398 (n=11 for Con; n=10 for NS398; panel C). The aortic rings in different protocols within panels are paired; the Up_4A concentration responses for each

control are similar to those within figures or in other figures. Values are means ± SEM. * $P < 0.05$, effect of drug vs. control (Con). * represents the concentration-response relation by two-way ANOVA.

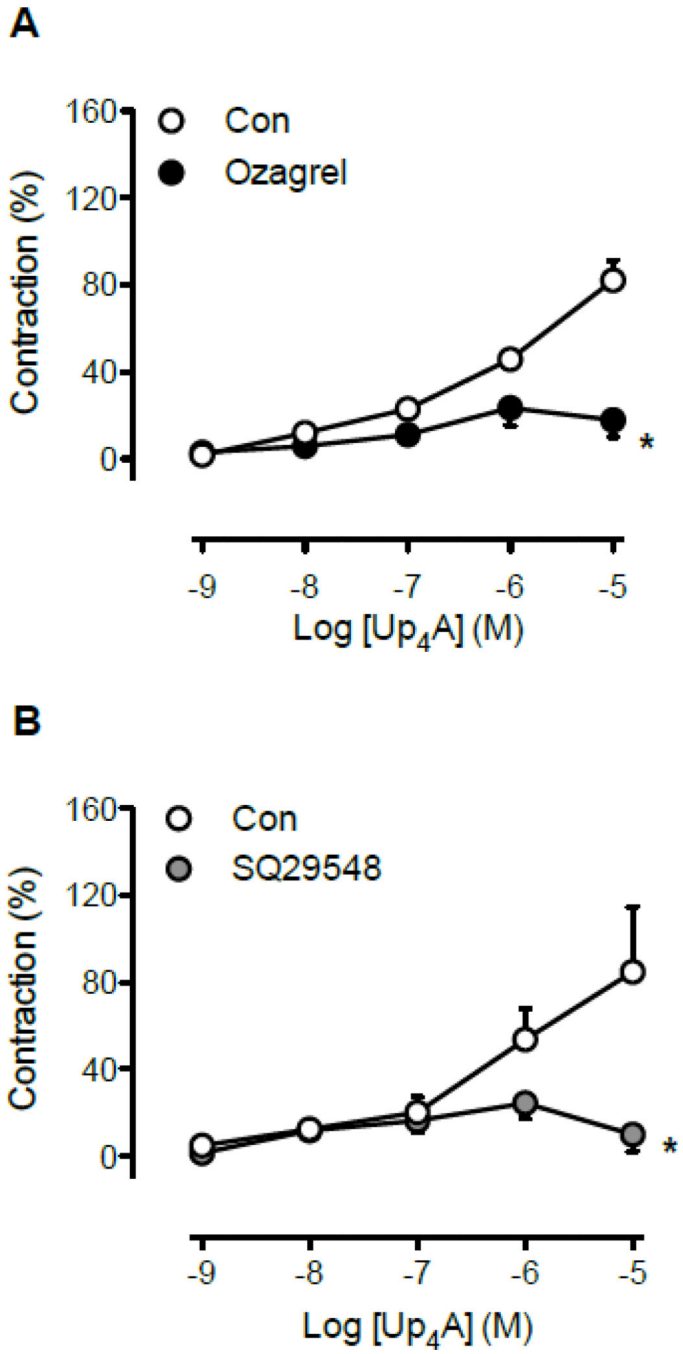

Figure 2.

Effects of thromboxane (TXA_2) synthase inhibition and TXA_2 receptor (TP) antagonism on Up_4A-induced vasoconstriction. Shown are concentration-responses to Up_4A in mouse aorta from WT in the absence and presence of 10 μM of the TX synthase inhibitor ozagrel (n=11 for both Con and ozagrel; panel A), and 1 μM of the TP antagonist SQ29548 (n=8 for Con; n=6 for SQ29548; panel B). The aortic rings in different protocols within panels are paired; the Up_4A concentration responses for each control are similar to those within figures or in

other figures. Values are means ± SEM. * $P < 0.05$, effect of drug vs. Con. * represents the concentration-response relation by two-way ANOVA.

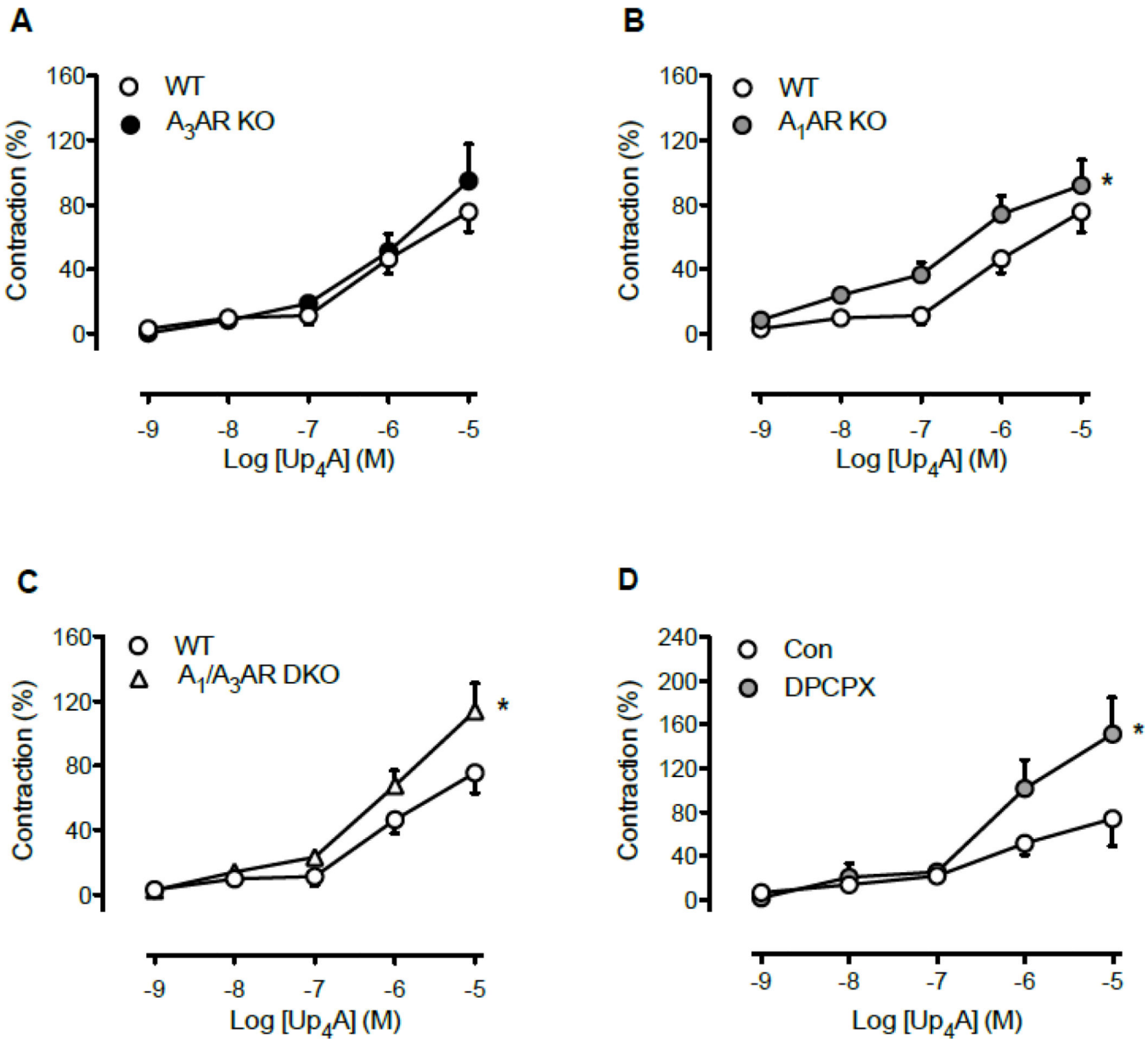

Figure 3.

Effects of A_1 adenosine receptor (AR) deletion or antagonism, A_3AR deletion and combined A_1AR and A_3AR deletion on Up_4A-induced vasoconstriction. Shown are concentration-responses to Up_4A in mouse aorta from A_3AR knockout (KO) mice (n=15 in WT; n=6 in A_3AR KO; panel A), A_1AR KO mice (n=15 in WT; n=6 in A_1AR KO; panel B), and A_1/A_3AR double KO (DKO) mice (n=15 in WT; n=11 in A_1/A_3AR DKO; panel C), as well as in mouse aorta from WT in the absence (n=10) and presence of 10 nM of selective A_1AR antagonist DPCPX (n=4; panel D). The aortic rings in different protocols within panels are paired; the Up_4A concentration responses for each control are similar to those within figures or in other figures. Values are means ± SEM. * $P < 0.05$ vs. Con or WT. * represents the concentration-response relation by two-way ANOVA.

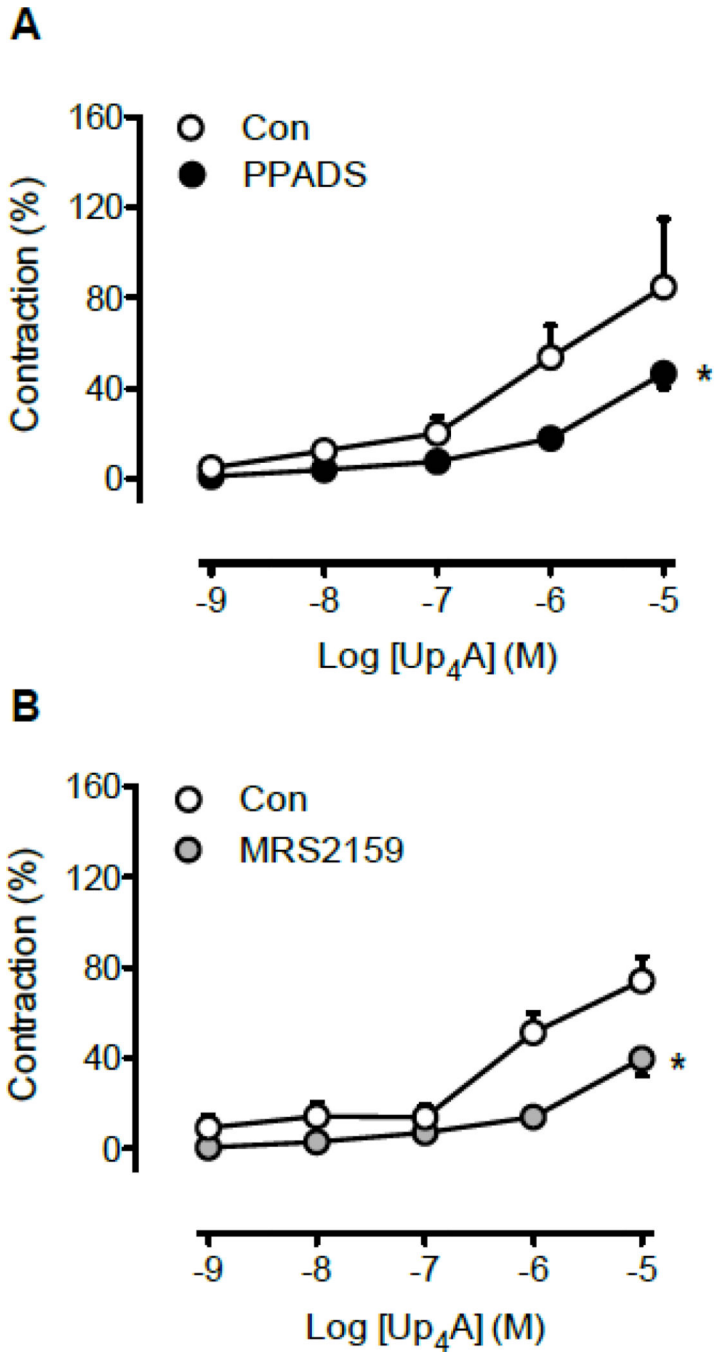

Figure 4.

Effects of non-selective purinergic P2 receptor (P2R) antagonism and selective $P2X_1R$ antagonism on Up_4A-induced vasoconstriction. Shown are concentration-responses to Up_4A in mouse aorta from WT in the absence and presence of 10 μM of non-selective P2R antagonist PPADS (n=8 for Con; n=7 for PPADS; panel A) and 30 μM of the selective $P2X_1R$ antagonist MRS2159 (n=6 for Con; n=7 for MRS2159; panel B). The aortic rings in different protocols within panels are paired; the Up_4A concentration responses for each control are similar to those within figures or in other figures. Values are means ± SEM. * P

< 0.05, effect of drug vs. Con. * represents the concentration-response relation by two-way ANOVA.

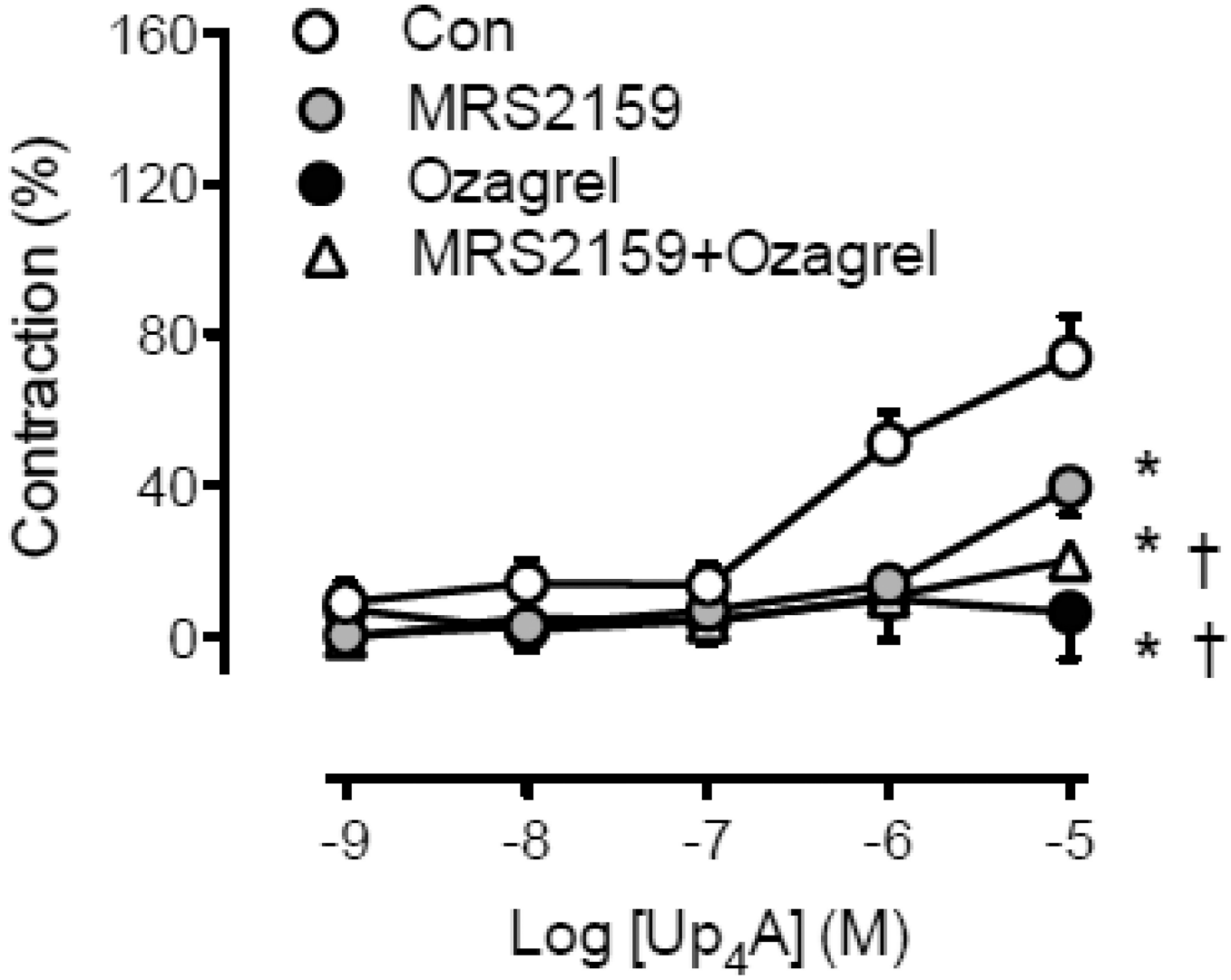

Figure 5.

Effects of $P2X_1R$ antagonism, TX synthase inhibition and combined $P2X_1R$ and TXA_2 inhibition. Shown are concentration-responses to Up_4A in mouse aorta from WT in the absence (n=6) and presence of 30 μM of the $P2X_1R$ antagonist MRS2159 (n=7), 10 μM of the TXA_2 antagonist ozagrel (n=6) and combine MRS2159 and ozagrel (n=6). The control curve and MRS2159 curve are the same as those in Fig. 4B. The aortic rings in different protocols in the figure are paired; the Up_4A concentration responses for control are similar to those in other figures. Values are means ± SEM. * $P < 0.05$ vs. Con; † $P < 0.05$ vs. MRS2159. * represents the concentration-response relation by two-way ANOVA.

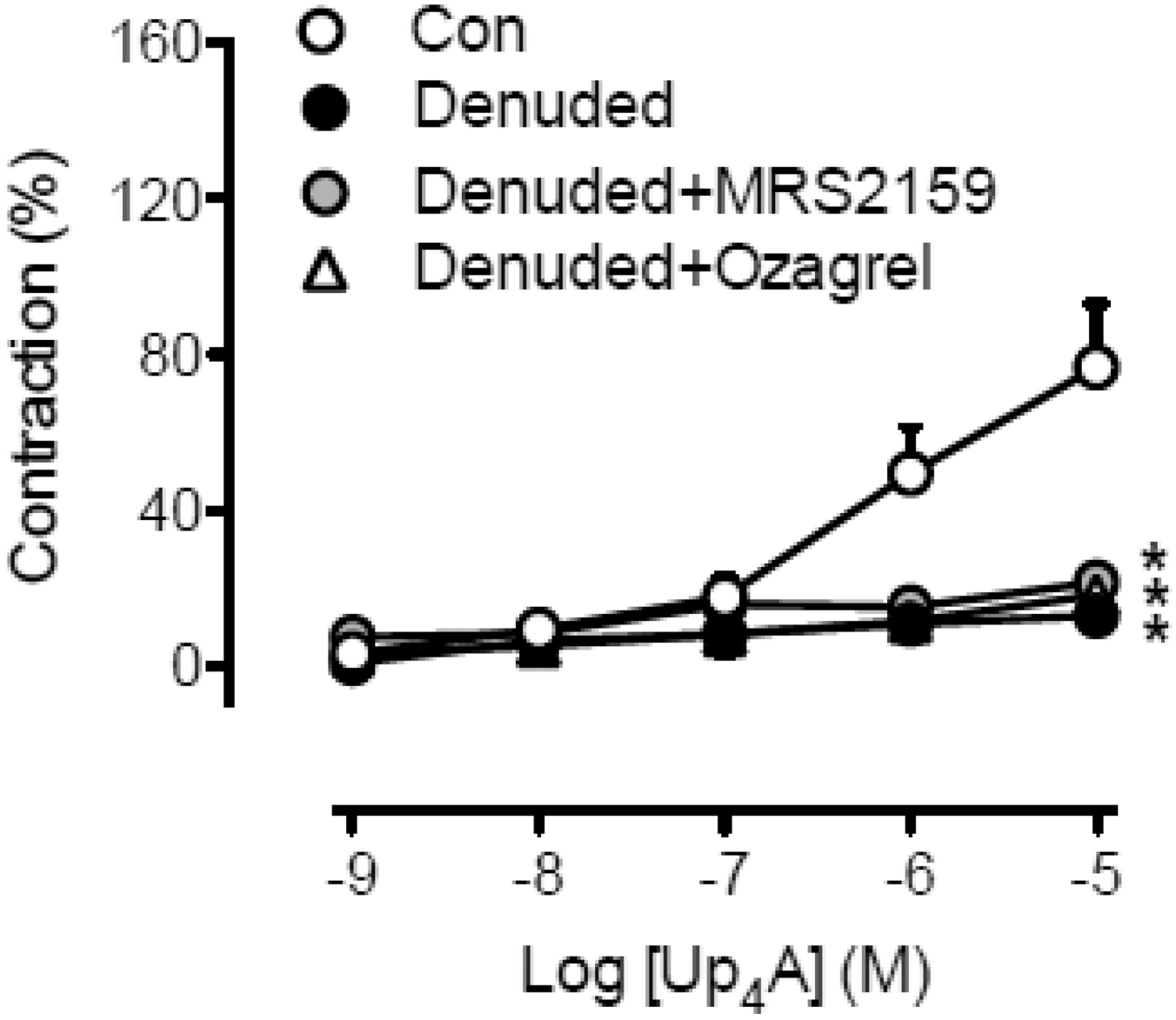

Figure 6.

Endothelium-dependency of vasoconstriction induced by Up_4A. Shown are concentration-responses to Up_4A in endothelium-non-denuded (n=7), endothelium-denuded rings and endothelium-denuded aortic rings from WT in the absence (n=5) and presence of 30 μM of the selective $P2X_1R$ antagonist MRS2159 (n=5) or 10 μM of the TX synthase inhibitor ozagrel (n=7). The aortic rings in different protocols in the figure are paired; the Up_4A concentration responses for control are similar to those in other figures. Values are means ± SEM. * $P < 0.05$ vs. Con. * represents the concentration-response relation by two-way ANOVA.

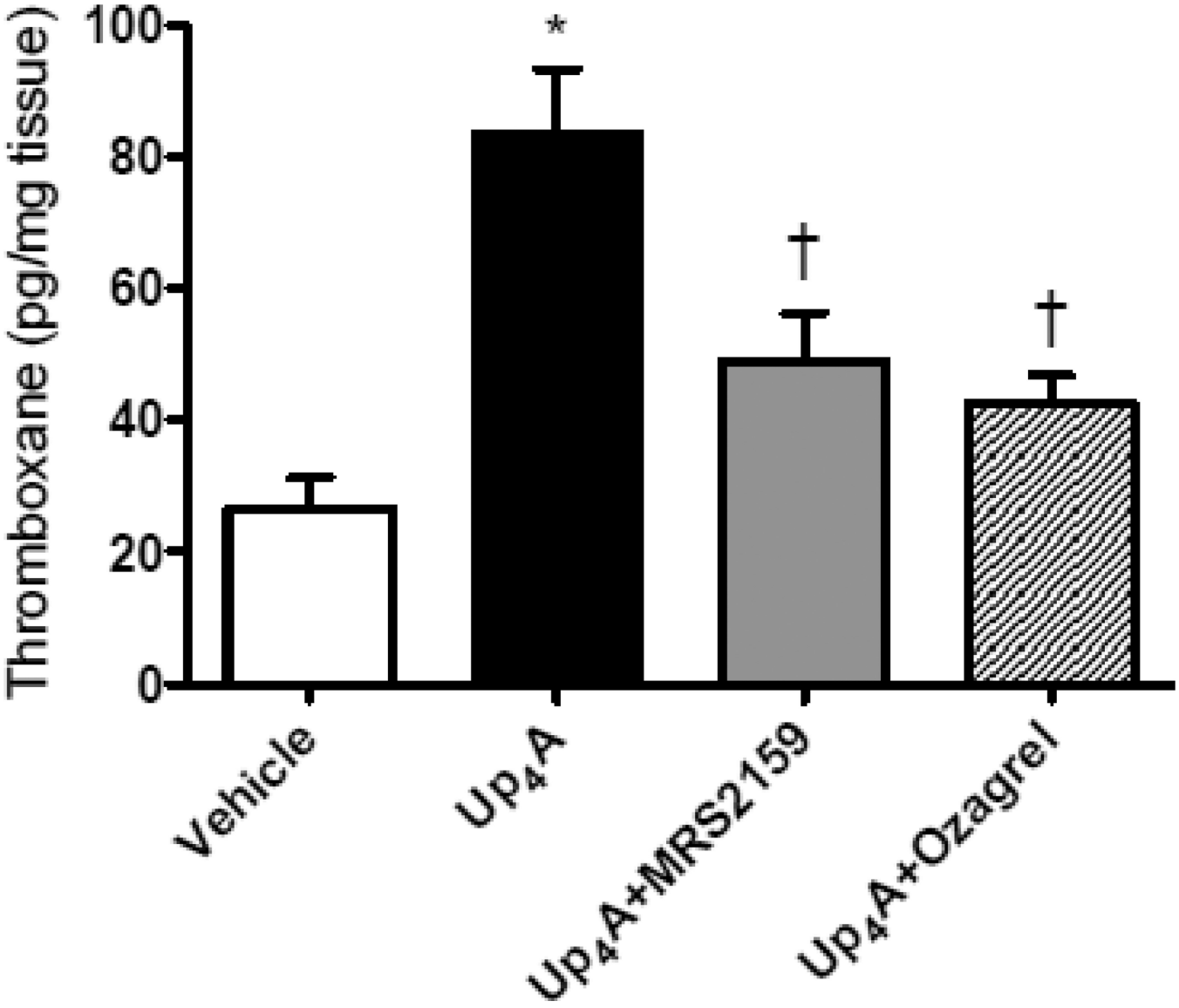

Figure 7.

Effects of Up_4A on TXA_2 production. Shown are effects of Up_4A (3 μM) on production of TXB_2, a metabolite of TXA_2, in intact isolated aortic rings from WT in the absence and presence of 30 μM of the selective $P2X_1R$ antagonist MRS2159 or 10 μM of the TX synthase inhibitor ozagrel. N=6 in each group. Values are means ± SEM. * $P < 0.05$ vs. Vehicle; † $P < 0.05$ vs. Up_4A by one-way ANOVA.