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Alterations in vasoconstrictor responses to the endothelium-derived contracting factor uridine adenosine tetraphosphate are region specific in DOCA-salt hypertensive rats

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Abstract

Uridine adenosine tetraphosphate (Up₄A) has been recently identified as a novel and potent endothelium-derived contracting factor and contains both purine and pyrimidine moieties, which activate purinergic P2X and P2Y receptors. The present study was designed to compare contractile responses to Up₄A and other nucleotides such as ATP (P2X/P2Y agonist), UTP (P2Y₂/P2Y₄ agonist), UDP (P2Y₆ agonist), and α,β -methylene ATP (P2X₁ agonist) in different vascular regions [thoracic aorta, basilar, small mesenteric, and femoral arteries] from deoxycorticosterone acetate-salt (DOCA-salt) and control rats. In DOCA-salt rats [vs. control uninephrectomized (Uni) rats]: (1) in thoracic aorta, Up₄A-, ATP-, and UTP-induced contractions were unchanged; (2) in basilar artery, Up₄A-, ATP-, UTP- and UDP-induced contractions were increased, and expression for P2X₁, but not P2Y₂ or P2Y₆ was decreased; (3) in small mesenteric artery, Up₄A-induced contraction was decreased and UDP-induced contraction was increased; expression of P2Y₂ and P2X₁ was decreased whereas P2Y₆ expression was increased; (4) in femoral artery, Up₄A-, UTP-, and UDP-induced contractions were increased, but expression of P2Y₂, P2Y₆ and P2X₁ was unchanged. The α,β -methylene ATP-induced contraction was bell-shaped and the maximal contraction was reached at a lower concentration in basilar and mesenteric arteries from Uni rats, compared to arteries from DOCA-salt rats. These results suggest that Up₄A-induced contraction is heterogeneously affected among various vascular beds in arterial hypertension. P2Y receptor activation may contribute to enhancement of Up₄A-induced contraction in basilar and femoral arteries. These changes in vascular reactivity to Up₄A may be adaptive to the vascular alterations produced by hypertension.

Keywords

contraction; dinucleotide; DOCA-salt; EDCF; extracellular nucleotides; P2 receptor

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Introduction

Arterial hypertension is associated with vasomotor dysfunction, which manifests in part as decreased endothelium-dependent relaxation and also as altered vascular smooth muscle function [1–5]. Although endothelium-derived relaxing factors (EDRFs) play a crucial role in the regulation of circulatory vasomotion, experimental evidence supports the concept that endothelium-derived contracting factors (EDCFs) also regulate vascular function, which becomes particularly important in the hypertensive process [2,3]. Impaired endothelium-dependent vasomotor function in hypertensive animal models has been attributed to both reduced nitric oxide (NO) bioavailability and enhanced endothelium-dependent contraction [2,3,6]. Alterations in the bioavailability and actions of endothelium-derived factors may contribute to changes in resting blood pressure. Although a strategy for suppressing endothelial dysfunction (i.e., increasing EDRFs signaling and/or reducing EDCF signaling) may become a therapeutic target for hypertensive disease, the detailed mechanisms by which endothelial factors regulate the cardiovascular system are not fully understood.

The dinucleotide uridine adenosine tetraphosphate (Up₄A) is a novel potent non-peptidic EDCF that is assumed to play an important role in the regulation of vascular tone [7], and may be a potent inducer of pro-inflammatory responses in the vascular wall [8]. Up₄A is released from the endothelium in response to acetylcholine (ACh), the calcium ionophore (A23187), endothelin-1, adenosine triphosphate (ATP), and uridine triphosphate (UTP) [7]. Since the identification of Up₄A in 2005 [7], Up₄A has been shown to modulate vascular tone in rat aorta [9], pulmonary arteries [10], and perfused kidney [7,11], and aorta [12] and renal afferent arterioles [13] isolated from mouse. Up₄A possesses both purine and pyrimidine moieties, which are known to potentially activate purinoceptors.

Purinoceptors are present in most organ systems in the body, and there is accumulating evidence that they play an important role in contractile tone of various arteries including cerebral arteries [14,15], thoracic aorta [16], mesenteric [17,18] and femoral arteries [19]. Purinoceptors have been classified into two subtypes: P1 and P2 receptors, based on their pharmacological properties and molecular cloning [20]. Adenosine and its phosphate derivatives, ATP and ADP, have been identified as the endogenous ligands for P1 and P2 receptors, respectively [20]. The P2 receptors are divided into two families: such as P2X (ligand-gated ion channels) and P2Y receptors (G protein-coupled receptors) [20–24]. So far, seven P2X receptor subtypes (P2X₁–P2X₇) and eight P2Y receptor subtypes (P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁–P2Y₁₄) have been reported [20–24]. P2X receptors are exclusively activated by ATP, whereas P2Y receptors respond to both purine (ATP and ADP) and pyrimidine (UTP and UDP) nucleotides [20–24]. Specifically, ATP is a ligand for P2X₁–P2X₇, P2Y₂, P2Y₁₁, and P2Y₁₃ receptors, whereas UTP is a ligand for P2Y₂ and P2Y₄ receptors [20–24]. UDP preferentially activates P2Y₆ receptors [25,26].

So far, the studies of Up₄A-mediated responses have been carried out only in normal animals, and there is no study to indicate the vascular effects of Up₄A under pathophysiological conditions, such as arterial hypertension. We recently found that Up₄A-induced contraction is increased in renal artery but not in pulmonary artery isolated from deoxycorticosterone acetate (DOCA)-salt rats, a salt-dependent experimental model of arterial hypertension [27]. However, regional specific differences of Up₄A and purinoceptor signaling in arterial hypertension are largely unknown/unexplored. Since circulating levels of Up₄A are strongly associated with juvenile hypertension [28], Up₄A may contribute to the early development of primary hypertension and to the development of hypertension-associated vascular abnormalities.

Hypertension leads to vascular complications in various regions including cerebrovascular [29,30] and peripheral vasculatures [31,32]. For instance, chronic hypertension leads to alteration of vascular responses both in resistance (e.g. small mesenteric artery, which plays an important role in blood pressure regulation [33,34]) and conductance arteries (e.g. basilar artery, which may make important contributions to total cerebral vascular resistance and is a major determinant of local microvascular pressure [35,36], thoracic aorta [3,4,33,37], femoral artery, which supplies blood to skeletal muscles of the lower extremity). Purinoceptor signaling may play an important role in regulation of vascular tone [14–19, 38]. However, the effects of arterial hypertension on Up₄A arterial responsiveness are yet to be determined.

For the present study, we hypothesized that regional differences in Up₄A-induced vasoconstriction would be identified in hypertension animals. To test this hypothesis, we designed experiments to characterize regional differences to Up₄A in DOCA-salt rats, a salt-dependent experimental model of arterial hypertension [33,37,39]. Basilar, thoracic aorta, small mesenteric and femoral arteries were used based on previous reports showing that hypertension leads to altered reactivity in these vessels and their heterogenous contribution to total peripheral resistance as well as their importance for local control of blood flow. Responses to Up₄A and other purinoceptor agonists were determined in these four different arteries from DOCA-salt rat and its control uninephrectomized (Uni) rats. Mechanisms involved in differential vascular regional responses to Up₄A were also investigated.

2. Materials and methods

2.1. Drugs and solutions

Acetylcholine (ACh), DOCA, phenylephrine (PE), ATP, UTP, UDP, and α,β -methylene ATP were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). Up₄A was obtained from Biolog Life Science Institute (Bremen, Germany). Drugs were dissolved in sterile HPLC grade water. The antibody against P2Y₂ was obtained from Abcam (Cambridge, MA, USA) and its respective blocking peptide was from Neuromics (Edina, MN, USA). The antibody against P2Y₆ and its respective blocking peptide were obtained from Enzo Life Sciences (Plymouth Meeting, PA, USA). The antibody against P2X₁ and its respective blocking peptide were obtained from Alomone Labs (Jerusalem, Israel).

2.2. Animals and experimental design

Male Wistar rats (8 weeks-old, 250–340 g; Harlan Laboratories, Indianapolis, IN, USA) were used in this study. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals and approved by the Georgia Health Sciences University Committee on the Use of Animals in Research and Education. The animals were housed two or three per cage on a 12-h light/dark cycle and fed a standard chow diet with water *ad libitum*.

DOCA-salt hypertension was induced in rats as previously described [35,36]. Briefly, all animals were uninephrectomized under anesthesia and were given no further treatment (Uni rats) or received 1% NaCl plus 0.2% KCl in the drinking water and DOCA silastic pellets (0.2 g/kg), which were implanted subcutaneously in the scapular region (DOCA-salt rats). The duration of treatment was 4 to 5 weeks.

One week before euthanasia, systolic blood pressure (SBP) was measured by the tail-cuff method using a CODA system (Kent Scientific Corporation). At the end of 4 to 5 weeks of treatment, basilar artery, thoracic aorta, second-order mesenteric artery, and femoral artery were removed and submitted to experimental procedures.

2.3. Measurement of isometric force

Vascular isometric force was recorded as previously described [33,37]. After euthanasia, basilar artery, thoracic aorta, femoral artery, and the mesentery were isolated and placed in an ice-cold physiologic salt solution (PSS) containing (mM): 130.0 NaCl, 4.7 KCl, 14.9 NaHCO₃, 5.5 glucose, 1.18 KH₂PO₄, 1.17 MgSO₄·7H₂O, 1.6 CaCl₂·2H₂O, and 0.026 EDTA. Each artery (thoracic aorta, basilar and femoral arteries, and second-order branches of mesenteric artery) was carefully cleaned of fat and connective tissue under a stereomicroscope. The arteries were then cut into 2 mm rings (several rings were obtained from each animal) and mounted on an isometric Mulvany-Halpen myograph (Danish MyoTech; Aarhus, Denmark) filled with 5 mL PSS and continuously gassed with 95% O₂-5% CO₂ while maintaining temperature at 37°C and recorded by a PowerLab 8/SP data acquisition system (ADInstruments). The rings were stretched until an optimal resting tension, which was established from preliminary length-active tension curves, of 2.0 mN (basilar artery), 30 mN (thoracic aorta), 2.3 mN (femoral artery), or 2.0 mN (mesenteric artery), and then allowed to equilibrate for at least 45 min. Arterial integrity was assessed by contracting the segments with a depolarizing concentration of potassium chloride (KCl, 120 mM) and subsequently, with PE (10⁻⁶ or 10⁻⁵ M) followed by relaxation with ACh (10⁻⁶ or 10⁻⁵ M).

After washing and a new stabilization, Up₄A (10⁻⁹ – 3×10⁻⁵ M), ATP (10⁻⁸ - 10⁻³ M), UTP (10⁻⁸ - 10⁻³ M), UDP (10⁻⁸ - 10⁻³ M) or α,β-methylene-ATP (10⁻⁸ – 3×10⁻⁵ M) was added cumulatively to the bath until a maximal response was achieved. After the addition of a chosen concentration of the agonist, a plateau response was allowed to develop before the addition of the next concentration.

In order to avoid the possibility of purinoceptor desensitization, rings were exposed to only one purinoceptor agonist. To investigate vasoconstriction to a receptor-independent stimulation, concentration-response curves to KCl (10 – 80 mM) were performed. Accordingly, after the concentration-response curve for a purinoceptor agonist, rings were washed several times with PSS until the tension returned to baseline. Subsequently, the concentration-response curve to KCl was constructed.

2.4. Western blotting for P2 receptor

The protein levels of P2Y₂, P2Y₆, and P2X₁ receptors were quantified using immunoblotting procedures, essentially as described before [27,33,37]. Basilar, mesenteric, and femoral arteries from DOCA-salt and Uni rats were isolated, cleaned from fat, dissected, and frozen in liquid nitrogen. Proteins [basilar artery: 5 μg (for P2X₁) or 15 μg (for P2Y₂ or P2Y₆), mesenteric artery: 20 μg, and femoral artery: 15 μg] extracted from each artery were separated by electrophoresis on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked with 5% skim milk in Tris-buffered saline solution with Tween (0.1%) for 1 h at 24°C. Membranes were then incubated with antibodies overnight at 4°C. Antibodies were as follows: anti-P2Y₂ (1:500; Abcam), anti-P2Y₆ (1:500; Enzo Life Sciences), anti- P2X₁ (1:500; alomone labs), and β-actin (1:15,000; Sigma). Specificity of the antibody was assessed using blocking peptides according to the manufacturer's instructions. After incubation with secondary antibodies, signals were revealed with chemiluminescence, visualized by autoradiography, and quantified densitometrically. Results were normalized by β-actin expression and expressed as a percentage of control. The β-actin expression was similar between DOCA-salt and Uni groups in each artery (data not shown).

2.5. Data analysis and statistics

Arterial contractions recorded from the myograph were expressed as changes in the displacement from baseline in mN and were represented as percent maximum contraction to 120 mM KCl (% contraction). The contractions induced by 120 mM KCl were similar between DOCA-salt and Uni groups in each group of arterial rings (Table 1). Agonist concentration-response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 5.0; GraphPad Software Inc., San Diego, CA, USA). Data are expressed as means \pm SEM. Statistical analysis of the concentration-response curves was performed by using 2-way analysis of variance (ANOVA) for comparison between the groups. When compared to control Uni group, data were analyzed using ANOVA with *post-hoc* Bonferroni testing or Student's *t*-test. Western blot data were analyzed by 1-sample *t* test. The values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. General parameters

At 3 or 4 weeks, DOCA-salt rats displayed higher systolic blood pressure in comparison with Uni rats (192 ± 3 mmHg, $n=34$ vs. 134 ± 2 mmHg, $n=37$, $P < 0.001$; respectively). At the time of the experiment, the body weight of the DOCA-salt rats was lower than that of Uni rats (333.2 ± 7.4 g, $n=34$ vs. 412.3 ± 4.9 g, $n=37$, $P < 0.001$; respectively).

3.2. Contractile response induced by isotonic K⁺

To investigate a contractile response induced by receptor-independent stimulation, we performed concentration-response curves to KCl, which is known to cause membrane depolarization and stimulate Ca²⁺ entry through voltage-gated Ca²⁺ channels [40]. As shown in Fig. 1, exposure of all arterial rings to isotonic high K⁺ solution (10–80 mM) led to a concentration-dependent increase in tension in the normotensive Uni and hypertensive DOCA-salt groups. The maximal contractile responses induced by high K⁺ (E_{max} ; % of 120 mM KCl-induced contraction) were similar in basilar artery [101.5 ± 5.6 %, ($n=9$) and 107.5 ± 5.9 %, ($n=7$), $P > 0.05$], thoracic aorta [98.8 ± 7.7 %, ($n=6$) and 116.4 ± 7.9 %, ($n=7$), $P > 0.05$], mesenteric artery [79.1 ± 2.2 %, ($n=6$) and 75.7 ± 3.5 %, ($n=6$), $P > 0.05$], and femoral artery [126.2 ± 7.8 %, ($n=6$) and 142.3 ± 10.7 %, ($n=5$), $P > 0.05$] from DOCA-salt and Uni rats, respectively. The EC_{50} values (mM) for the high K⁺-induced contractions were similar in basilar artery [35.5 ± 1.7 , ($n=9$) and 31.5 ± 2.5 , ($n=7$), $P > 0.05$], thoracic aorta [28.2 ± 1.6 , ($n=6$) and 25.6 ± 2.0 , ($n=7$), $P > 0.05$], mesenteric artery [38.4 ± 1.6 , ($n=6$) and 41.7 ± 1.7 , ($n=6$), $P > 0.05$], and femoral artery [36.6 ± 2.0 , ($n=6$) and 39.9 ± 0.02 , ($n=5$), $P > 0.05$] from DOCA-salt and Uni rats, respectively.

3.3. Contractile response induced by Up₄A

Cumulative administration of Up₄A (10^{-9} – 3×10^{-5} M) induced concentration-dependent contractions in all arterial rings from both DOCA-salt and Uni rats (Fig. 2). In DOCA-salt rats (vs. Uni rats), Up₄A-induced contractile response was significantly increased in basilar artery (Fig. 2A), unchanged in thoracic aorta (Fig. 2B), significantly decreased in mesenteric artery (Fig. 2C), and significantly increased in femoral artery (Fig. 2D).

3.4. Contractile response induced by other nucleotides

Since Up₄A contains both purine and pyrimidine moieties, we next investigated whether contractile responses induced by other nucleotides were altered in these four different arteries from DOCA-salt rats.

The contractile response induced by the maximal concentration of ATP (i.e., 10^{-3} M) was significantly augmented in basilar arteries from DOCA-salt rats compared to that in than in arteries from Uni rats (Fig. 3A). Exposure of thoracic aorta (Fig. 3B), mesenteric (Fig. 3C), and femoral arteries (Fig. 3D) to ATP produced very weak contractions, which were similar between the two groups.

The contractile response induced by UTP was significantly increased in basilar (Fig. 4A) and femoral (Fig. 4D) arterial rings from DOCA-salt rats compared to those in rings from Uni rats. On the other hand, UTP-induced contraction was similar in thoracic aorta (Fig. 4B) and mesenteric artery (Fig. 4C) between the two groups.

We further investigated the effects of UDP [endogenous P2Y receptor agonist that preferentially activates P2Y₆ [25,26] (Fig. 5)] and α,β -methylene ATP [selective P2X₁ agonist [41,42] (Fig. 6)] in basilar, mesenteric, and femoral arteries from DOCA-salt rats, which displayed altered responses to Up₄A. UDP-induced contraction was significantly greater in basilar (Fig. 5A), mesenteric (Fig. 5B), and femoral (Fig. 5C) arteries from DOCA-salt rats (vs. Uni rats). On the other hand, α,β -methylene ATP-induced contraction was similar in basilar (Fig. 6A) and femoral (Fig. 6C) arteries between the two groups. However, the maximal contractile response of α,β -methylene ATP-induced contraction was reached at a lower concentration in basilar and mesenteric arteries from Uni rats, compared to arteries from DOCA-salt rats (Figs. 6A,B). The concentration-response curve to α,β -methylene ATP was bell-shaped in basilar (Fig. 6A) and mesenteric (Fig. 6B) arteries.

3.5. Protein expression of P2 receptors

Conceivably, an alteration in P2 receptor expression could underlie the altered Up₄A-mediated constriction in basilar, mesenteric, and femoral arteries. Accordingly, we investigated P2 receptors protein expression in these arteries (Figs. 7–9). The P2Y₂ polyclonal antibody detected multiple bands, with two bands between 50 and 75 kDa in the basilar artery, two other bands between 37 and 50 kDa (one in mesenteric artery) or (two in basilar and femoral arteries), and one band corresponding to approximately 35 kDa. Multiple bands for P2Y₂ receptor have been reported in previous studies [43–45], and could result from posttranscriptional modification of protein, oligomerization of the receptor, or formation of heteromers with other proteins [44,45]. Importantly, the band between the 37 and 75 kDa disappeared, but the band at ~35 kDa remained when the primary antibody was pre-incubated with the appropriate antigenic peptide (Fig. 7). The bands of P2Y₆ (Fig. 8) and P2X₁ (Fig. 9) were completely lost using each blocking peptide (data not shown). Protein expressions of P2Y₂ (Fig. 7A; analyzed sum of four multiple bands, except ~35kDa) and P2Y₆ (Fig. 8A) were similar between basilar arteries from DOCA-salt and Uni rats. On the other hand, P2X₁ (Fig. 9A) receptor expression was significantly reduced in DOCA-salt basilar artery. Interestingly, an altered expression profile was observed in mesenteric artery from DOCA-salt rats (vs. Uni rats) as follows; reduced P2Y₂ (Fig. 7B) and P2X₁ (Fig. 9B), and increased P2Y₆ (Fig. 8B). In femoral artery, protein expression of P2Y₂ (Fig. 7C; analyzed sum of two multiple bands, except ~35kDa), P2Y₆ (Fig. 8C), and P2X₁ (Fig. 9C) receptors was similar between arteries from DOCA-salt and Uni rats.

4. Discussion

Earlier studies have demonstrated that the dinucleotide Up₄A produces constriction in various arteries from animals under normal conditions (viz. non-pathophysiological state) [7, 9–13]. In the present study, we have demonstrated that regional differences in Up₄A-mediated contraction are observed in DOCA-salt rats, a salt-dependent experimental model of arterial hypertension.

Purinergic receptors are present in most organ systems in the body, and there is accumulating evidence that they play an important role in contractile tone of arteries, as observed in the present study (viz. cerebral arteries [14,15], thoracic aorta [16], mesenteric arteries [17,18], and femoral arteries [19]). Several reports suggest that the activation of both P2X and P2Y receptors on smooth muscle cells leads to constriction in various arteries [21–23]. However, the relative importance of different P2 receptor subtypes in vascular smooth muscle for contraction is complex and delineation of the contributions of specific subtypes is complicated by the lack of highly selective agonists/antagonists. Available data suggest that ionotropic P2X₁ receptors and G protein-coupled P2Y₂, P2Y₄, and P2Y₆ receptors mainly contribute to nucleotide-mediated contraction [21–23]. Up₄A has both purine and pyrimidine moieties, which can potentially activate purinergic receptors. In rat pulmonary artery, Up₄A leads to contraction via P2Y receptors [10]. In perfused rat kidney, Up₄A-induced vasoconstriction depends not only on the activation of the P2X₁ receptor [7] but also on the activation of the P2Y₂ receptor [11]. Therefore, in the present study, we investigated whether DOCA-salt hypertension is associated with abnormal Up₄A-induced vasoconstriction. Vasoconstriction activated by other purinoceptor ligands such as ATP (P2X/P2Y agonist), UTP (P2Y₂/P2Y₄ agonist), UDP (P2Y₆ agonist), and α,β -Me ATP (P2X₁ agonist), and vascular purinoceptor expression (viz. P2Y₂, P2Y₆, and P2X₁) were also examined.

In the thoracic aorta, the Up₄A-induced contraction was similar between DOCA-salt hypertensive and normotensive Uni rats. Moreover, ATP- and UTP-induced contractions were also similar between the two groups. These results suggest that the contractile responses induced by P2 receptor activation are not influenced by chronic systemic hypertension in this representative large conduit artery.

We found that Up₄A-induced contraction was increased in basilar and femoral arteries, whereas contraction to Up₄A was reduced in mesenteric arteries from DOCA-salt hypertensive rats, compared to Uni rats. To further investigate vascular purinoceptor signaling, we examined contractile responses induced by ATP, UTP, UDP, and α,β -Me ATP. Pharmacological studies coupled with expression of cloned receptors in rats indicate that P2Y₂ and P2Y₄ are selective for ATP and UTP, whereas P2Y₆ favors UDP [17,46]. α,β -Me ATP is a full agonist at arterial smooth muscle P2X receptors and responses are rapidly inactivated in the continued presence of the agonist [47]. This native phenotype closely resembles that of recombinant P2X₁ receptors, which are expressed at high levels by arterial smooth muscle cells [48]. In the present study, we found that in basilar, mesenteric, and femoral arteries from DOCA-salt hypertensive rats (vs. Uni group): that 1) ATP-induced contraction, which induces a relatively weak in accordance to previous reports [10,49], is increased in basilar artery, but not in mesenteric and femoral arteries, 2) UTP-induced contraction is increased in basilar and femoral arteries, but not in mesenteric artery, 3) UDP-induced contraction is increased in basilar, mesenteric, and femoral arteries, and 4) higher concentrations of α,β -Me ATP are needed to contraction in mesenteric arteries, whereas contraction induced by α,β -Me ATP is not changed in femoral artery. These results suggest that enhanced Up₄A-induced contraction in basilar and femoral arteries from hypertensive rats may be attributable to P2Y signaling rather than P2X₁ signaling.

In the present study, we found in DOCA-salt group (vs. Uni group) that 1) receptor expression of P2Y₂ and P2Y₆ did not change, although P2X₁ receptor expression was decreased in basilar artery, 2) downregulation of P2Y₂ and P2X₁ and upregulation of P2Y₆ were seen in mesenteric artery, and 3) the receptor expression of P2Y₂, P2Y₆, and P2X₁ did not change in femoral artery. These results suggest that the enhancement of Up₄A-induced contraction in basilar and femoral arteries may alter down-stream pathways upon P2Y receptor stimulation rather than receptor expression. Various down-stream pathways (which

are related to vasoconstriction) are activated by P2Y receptor stimulation, such as MAPK and Rho kinase [8,50]. Moreover, several reports have demonstrated that these signaling pathways are altered in arteries from DOCA-salt hypertensive animals [5,35,51]. Indeed, our recent study showed that enhanced Up₄A-induced contraction in DOCA-salt renal artery was reduced by an ERK pathway inhibitor, and Up₄A-stimulated ERK activation was increased in renal artery from DOCA-salt rats [27].

In contrast to enhanced Up₄A-induced contraction in basilar and femoral arteries, a reduced response to Up₄A was observed in mesenteric artery from DOCA-salt rats. Although downregulation of P2Y₂ and P2X₁ may partially contribute to the reduced Up₄A-induced contraction in mesenteric artery from DOCA-salt rats, other receptor(s), including an unknown receptor for Up₄A may also contribute to this response, since ATP- and UTP-induced contractions were similar in mesenteric arteries from DOCA-salt and Uni rats. Since augmented P2Y₆ signaling (UDP-induced contraction and P2Y₆ upregulation) was seen in DOCA-salt mesenteric artery, decreased Up₄A-induced response is not due to decreased P2Y₆-mediated signaling. The existence of specific dinucleotide phosphate receptors has been proposed [52,53]. However, the specificity of dinucleotide receptors and their implication into multiple extracellular signals are still beginning to be understood.

In the present study, we demonstrated that arterial hypertension is associated with heterogeneous Up₄A-induced arterial contraction. The total peripheral resistance (TPR) is increased in the DOCA-salt rat model [54]. In the present study, Up₄A-induced contraction was similar in thoracic aorta, which has a small contribution to TPR, from control and DOCA-salt rats. On the other hand, in small mesenteric artery (which greatly contributes to TPR), Up₄A-induced contraction was lower in the DOCA-salt group. Considering our data, we speculate that the vasoconstriction induced by this dinucleotide does not contribute to increased peripheral resistance in this hypertensive model. Arterial hypertension is one of the main risk factors for cerebrovascular diseases [55]. In the cerebral circulation, large arteries such as the basilar artery make important contributions to total cerebral vascular resistance and are major determinants of local microvascular pressure [35]. On the other hand, the femoral arteries supply blood to the muscles of the lower extremities, which may play an important role in regulation of the peripheral arterial resistance. Enhanced Up₄A-induced contraction in these arteries may contribute to the increased resistance of cerebral and peripheral circulation during chronic arterial hypertension. Up₄A, therefore, potentially plays an important role in the regulation of vascular tone. Further study using other experimental models of arterial hypertension (e.g. angiotensin II-induced hypertension), and at various stages of hypertension (initial and established phases), as well as additional *in vitro* and *in vivo* techniques, will lead to a better understanding of the pathophysiological roles of Up₄A.

There is a limitation of the present study that should be mentioned. The concentrations of extracellular nucleotides are regulated by ectonucleotidases (e.g., nucleoside triphosphate diphosphohydrolase-1 and -2) and these enzymes are expressed in the vasculature [56, 57]. The enzymatic breakdown of dinucleoside polyphosphates leads to generation of mononucleotides and nucleotides that, in turn, are biologically active. A possible interpretation of the present results is that metabolic inter-conversion of nucleotides may be altered in the vasculature of hypertensive animals. Indeed, there is evidence that ecto-ATPase activity is increased in platelets from hypertensive patients [58]. However, the relationship between purinergic ligand-receptor interaction (activation) and vascular tone is complex since not only dinucleotide but also its metabolites can affect vascular tone. Further studies will be required to investigate these points.

5. Conclusions

In summary, the present results suggest that the existence of vascular regional differences in Up₄A-induced contraction in DOCA-salt hypertensive rats. Understanding the regulation of vascular tone by Up₄A and its signal-transduction may be of extreme value to understanding the pathophysiology of hypertension. We believe that our findings should stimulate further interest in the Up₄A system as a potential therapeutic target in hypertension-associated vascular dysfunctions.

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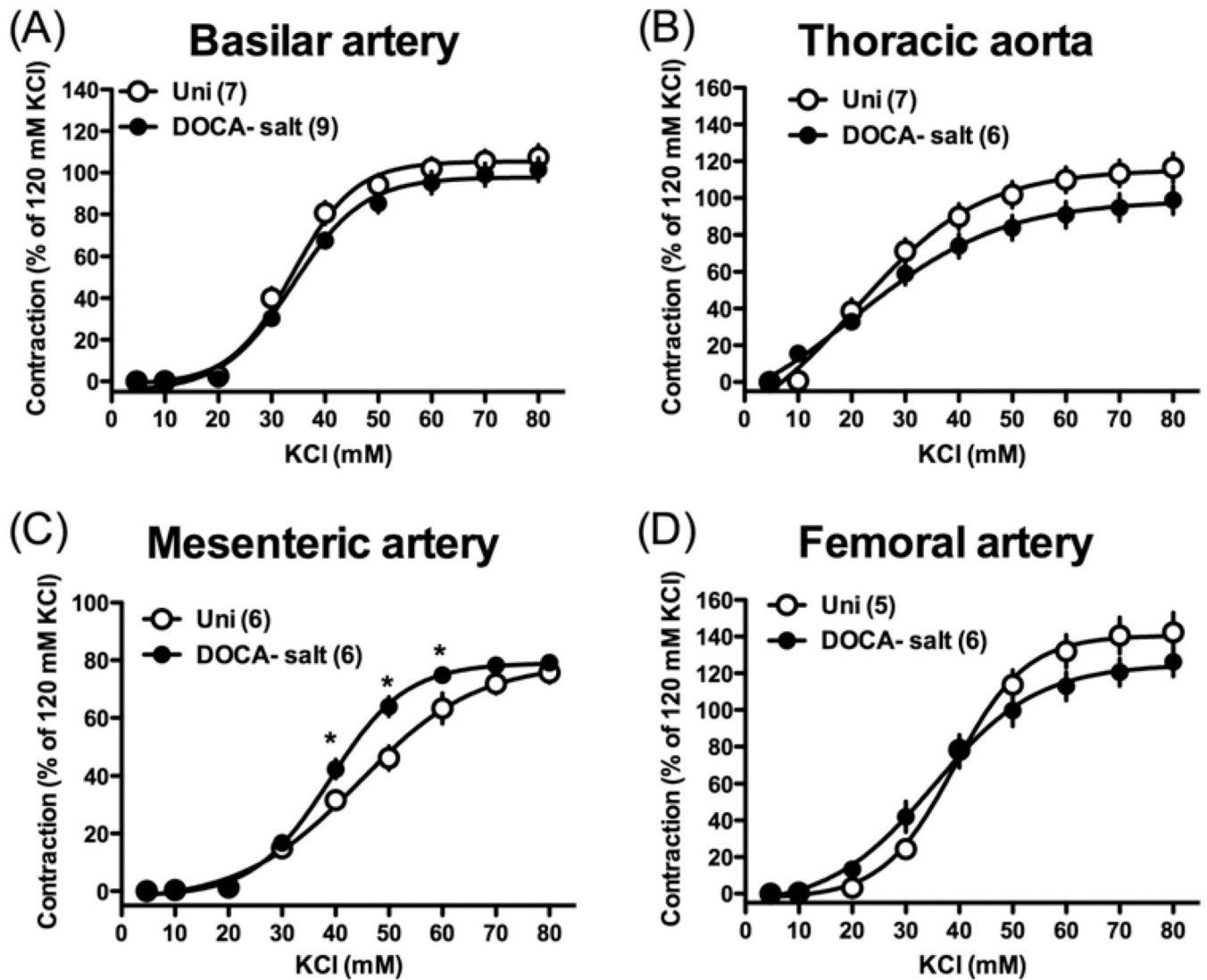


Fig. 1. Concentration-response curves for isotonic high-K⁺ solution in basilar artery (A), thoracic aorta (B), mesenteric artery (C), and femoral artery (D) from DOCA-salt hypertensive and control Uni rats. Data are means \pm SEM. Number of determinations is shown within parentheses. *P < 0.05, DOCA-salt vs. Uni group.

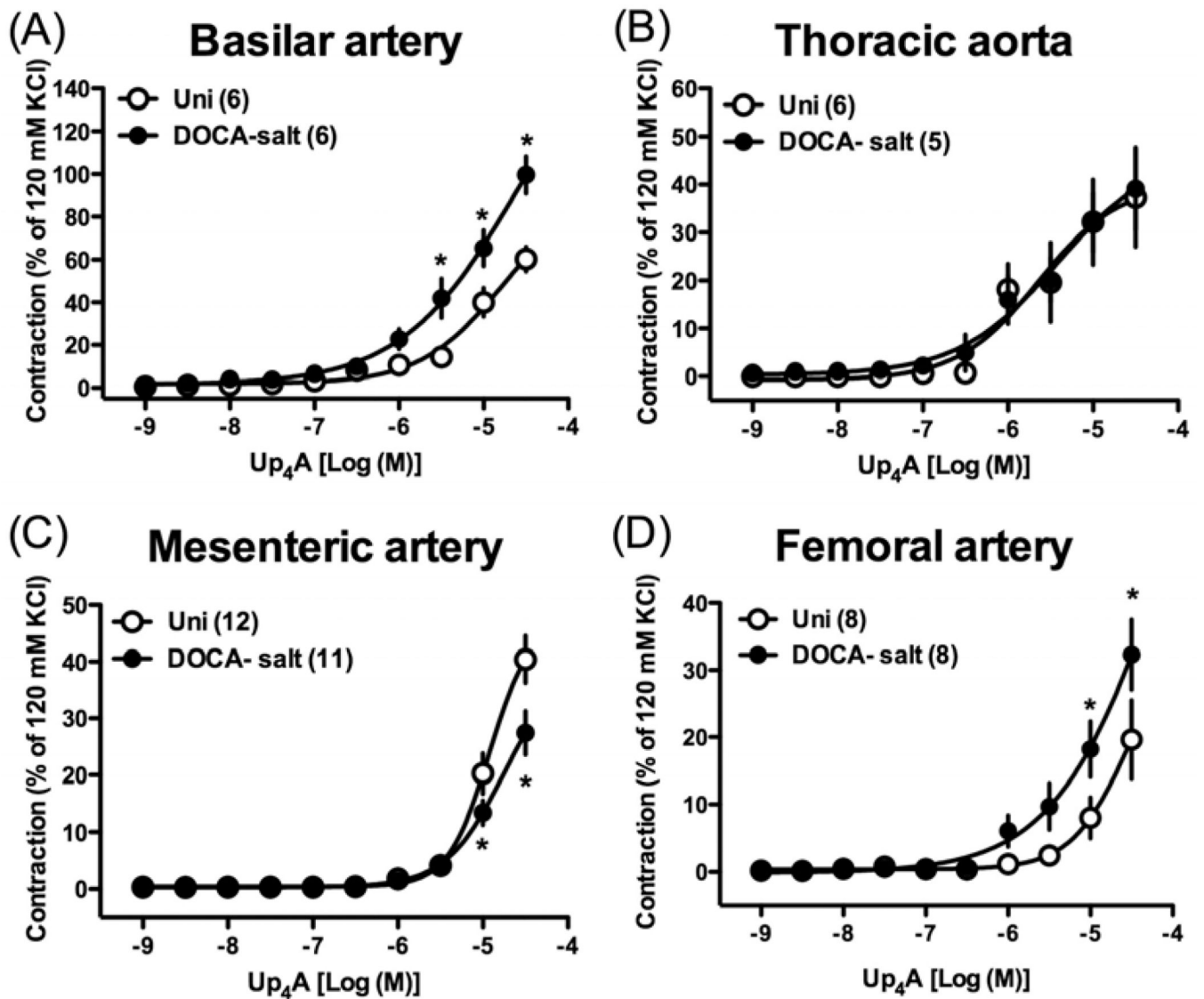


Fig. 2. Concentration-response curves for Up_4A in basilar artery (A), thoracic aorta (B), mesenteric artery (C), and femoral artery (D) from DOCA-salt hypertensive and control Uni rats. Data are means \pm SEM. Number of determinations is shown within parentheses. * $P < 0.05$, DOCA-salt vs. Uni group.

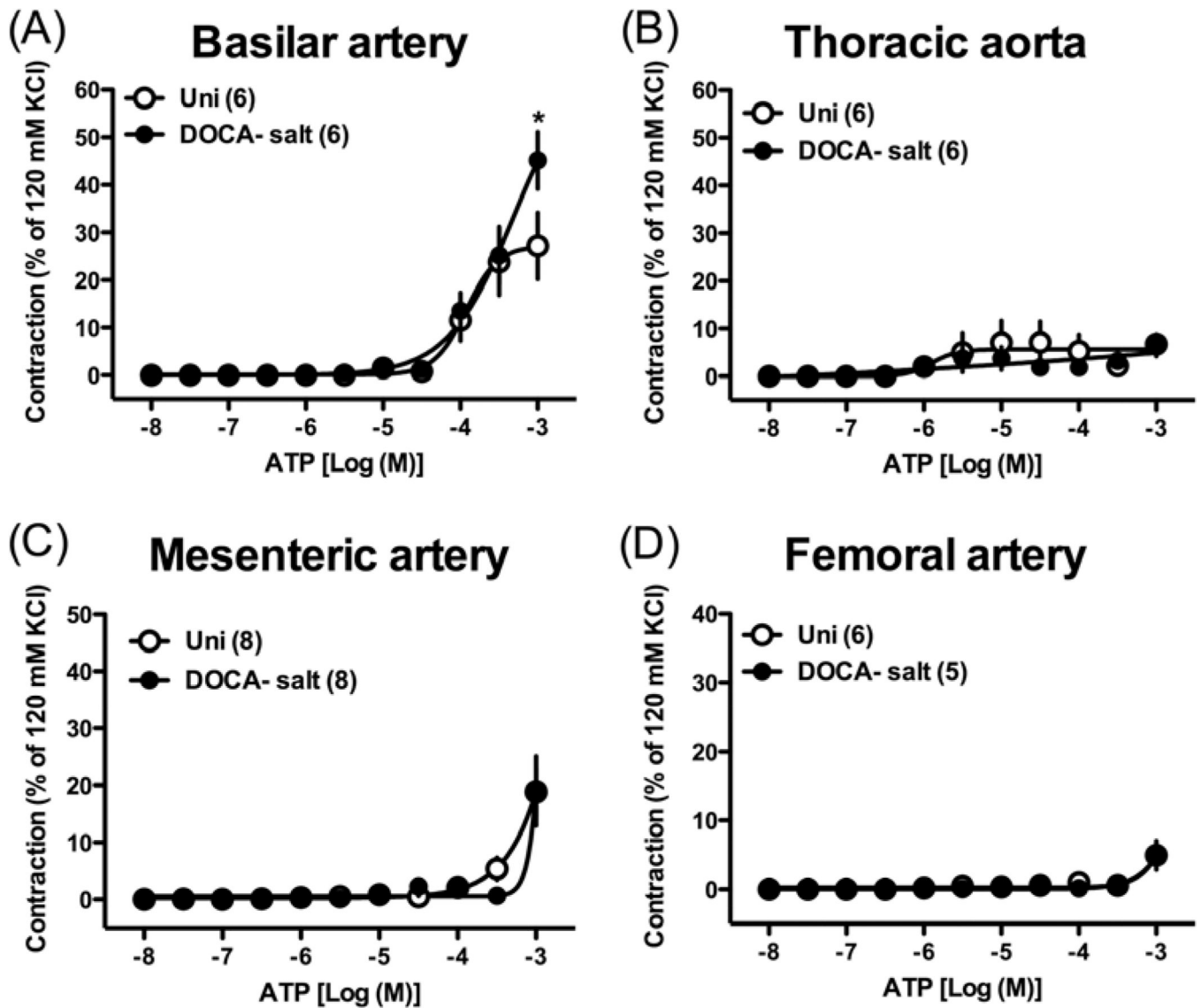


Fig. 3. Concentration-response curves for ATP in basilar artery (A), thoracic aorta (B), mesenteric artery (C), and femoral artery (D) from DOCA-salt hypertensive and control Uni rats. Data are means \pm SEM. Number of determinations is shown within parentheses. * $P < 0.05$, DOCA-salt vs. Uni group.

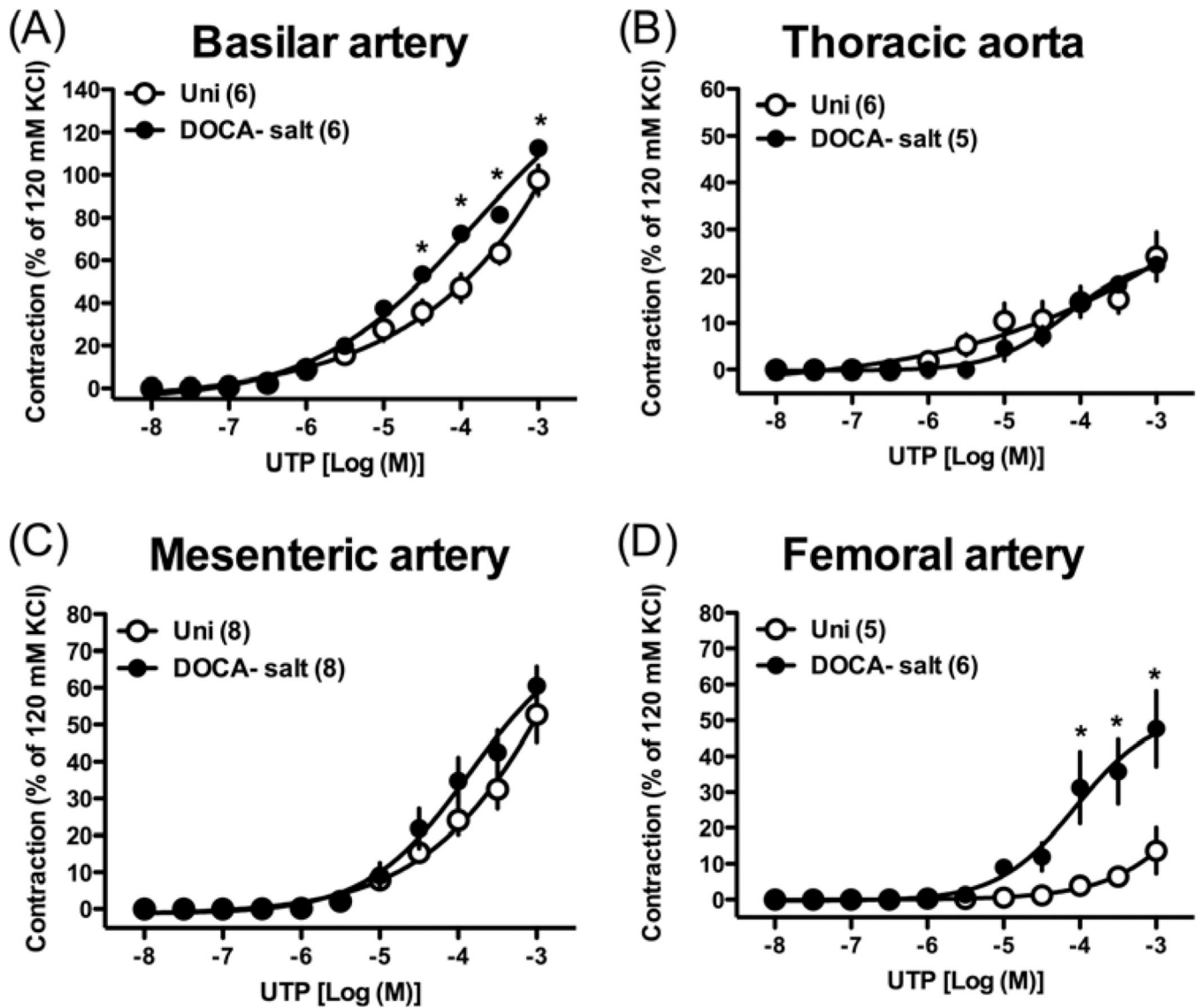


Fig. 4. Concentration-response curves for UTP in basilar artery (A), thoracic aorta (B), mesenteric artery (C), and femoral artery (D) from DOCA-salt hypertensive and control Uni rats. Data are means \pm SEM. Number of determinations is shown within parentheses. * $P < 0.05$, DOCA-salt vs. Uni group.

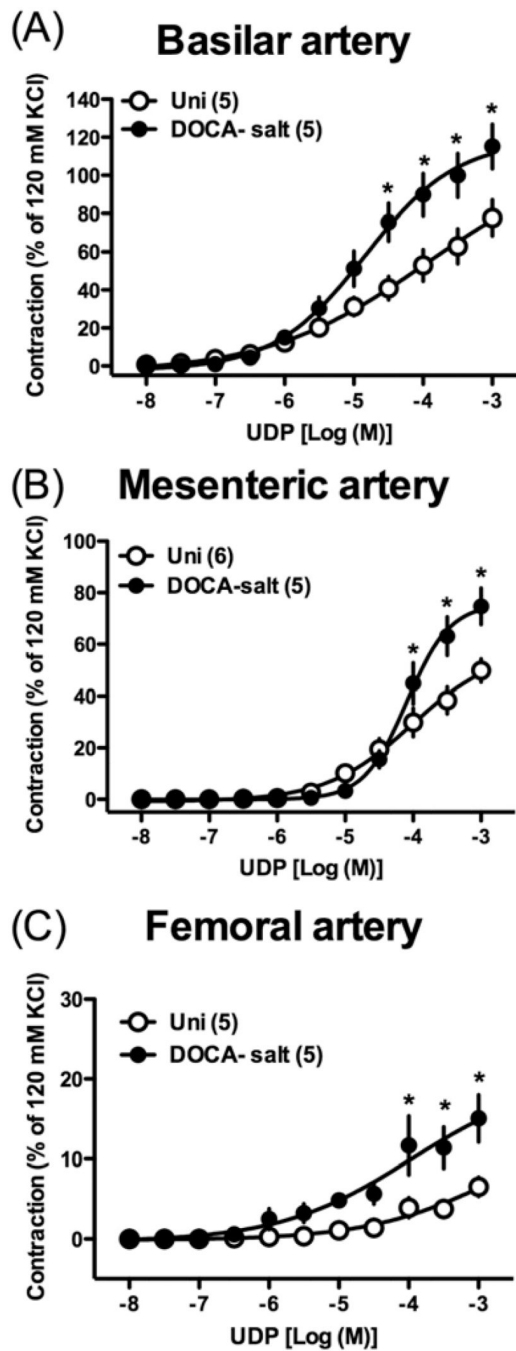


Fig. 5. Concentration-response curves for UDP in basilar artery (A), mesenteric artery (B), and femoral artery (C) from DOCA-salt hypertensive and control Uni rats. Data are means \pm SEM. Number of determinations is shown within parentheses. * $P < 0.05$, DOCA-salt vs. Uni group.

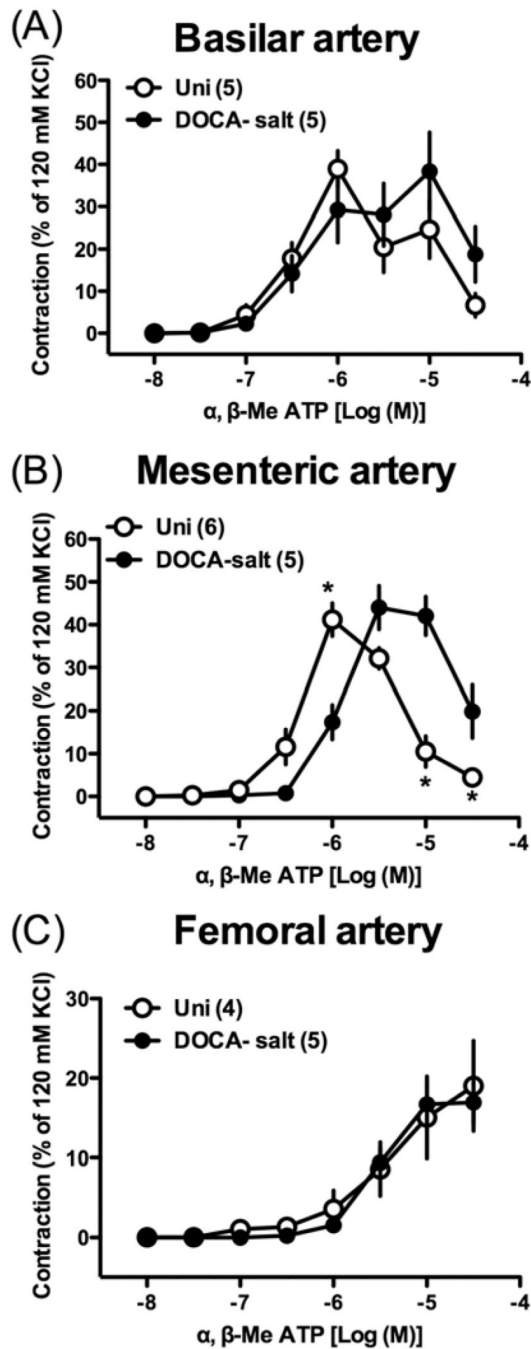
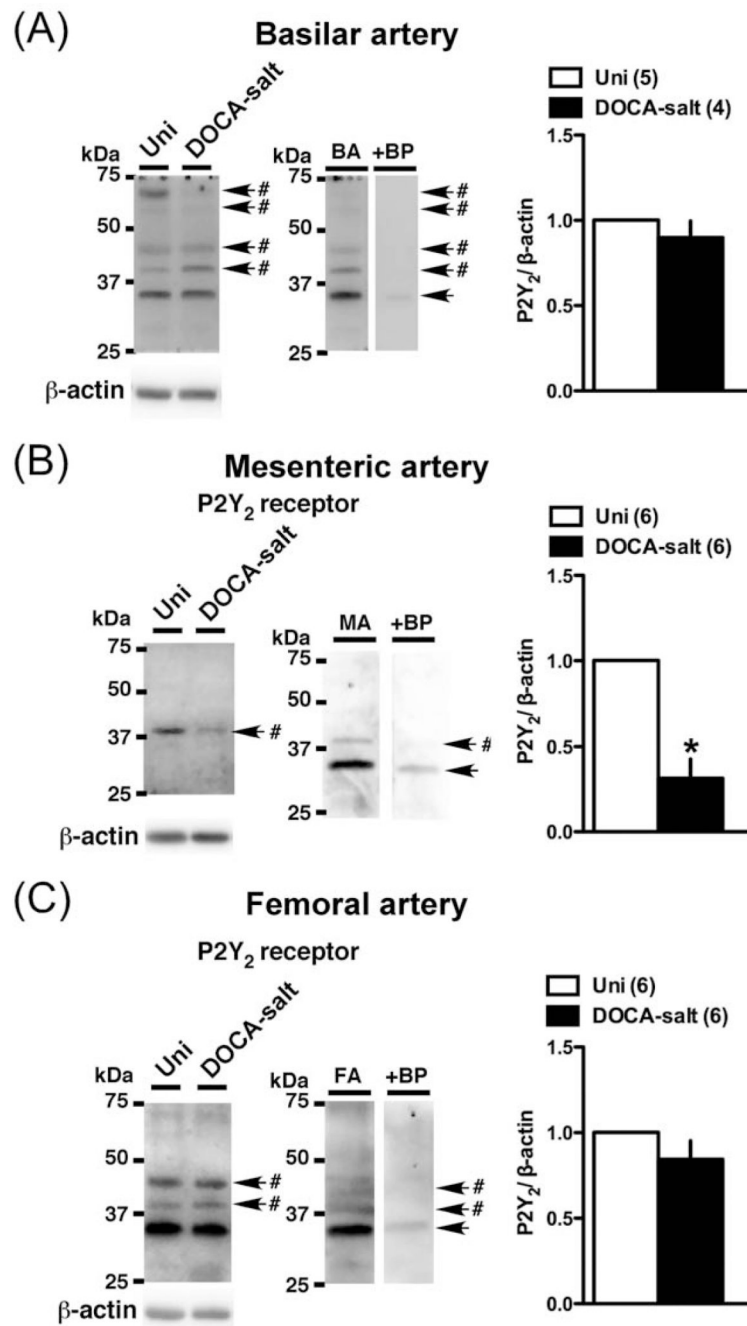


Fig. 6. Concentration-response curves for α,β -methylene ATP in basilar artery (A), mesenteric artery (B), and femoral artery (C) from DOCA-salt hypertensive and control Uni rats. Data are means \pm SEM. Number of determinations is shown within parentheses. * $P < 0.05$, DOCA-salt vs. Uni group.

**Fig. 7.**

Western blots for P2Y₂ receptor in basilar artery (A), mesenteric artery (B), and femoral artery (C) from DOCA-salt and Uni rats. *Left*: representative Western blots for P2Y₂ and β-actin. #Band disappeared after the incubation of the primary antibody with the respective blocking peptide. BP, blocking peptide. *Right*: bands were quantified as described in Materials and methods. Ratios were calculated for the optical density of each receptor over that of β-actin. Number of determinations is shown within parentheses. *P < 0.05, DOCA-salt vs. Uni group.

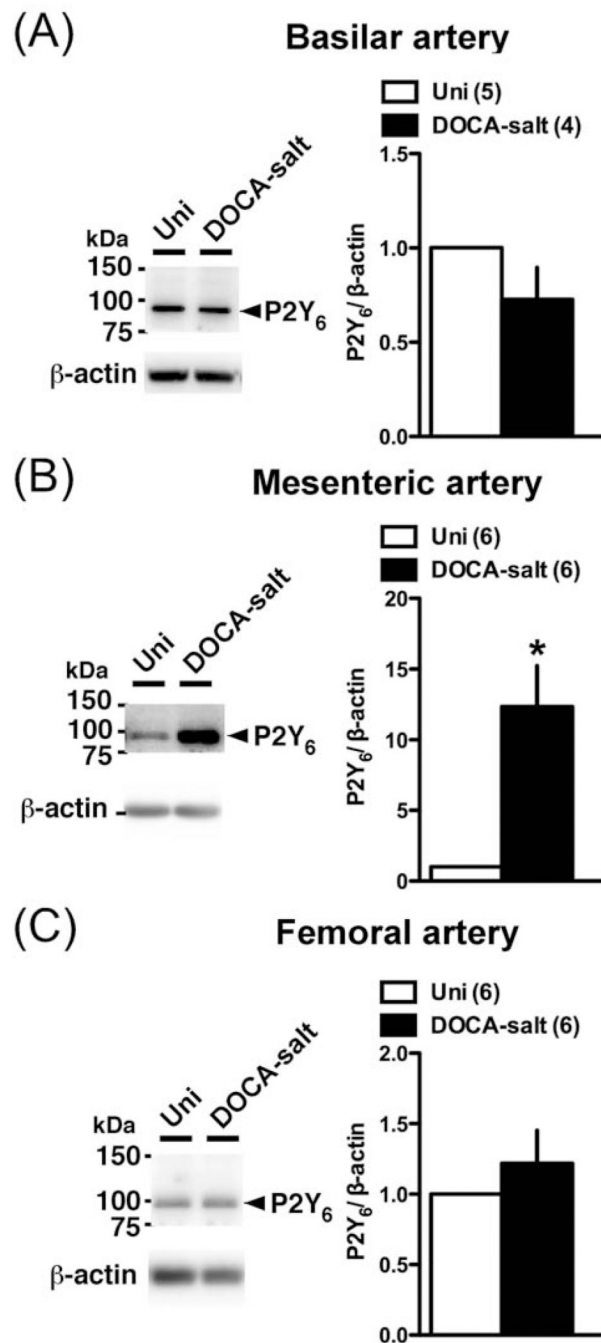


Fig. 8. Western blots for P2Y₆ receptor in basilar artery (A), mesenteric artery (B), and femoral artery (C) from DOCA-salt and Uni rats. *Left:* representative Western blots for P2Y₆ and β-actin. *Right:* bands were quantified as described in Materials and methods. Ratios were calculated for the optical density of each receptor over that of β-actin. Number of determinations is shown within parentheses. *P < 0.05, DOCA-salt vs. Uni group.

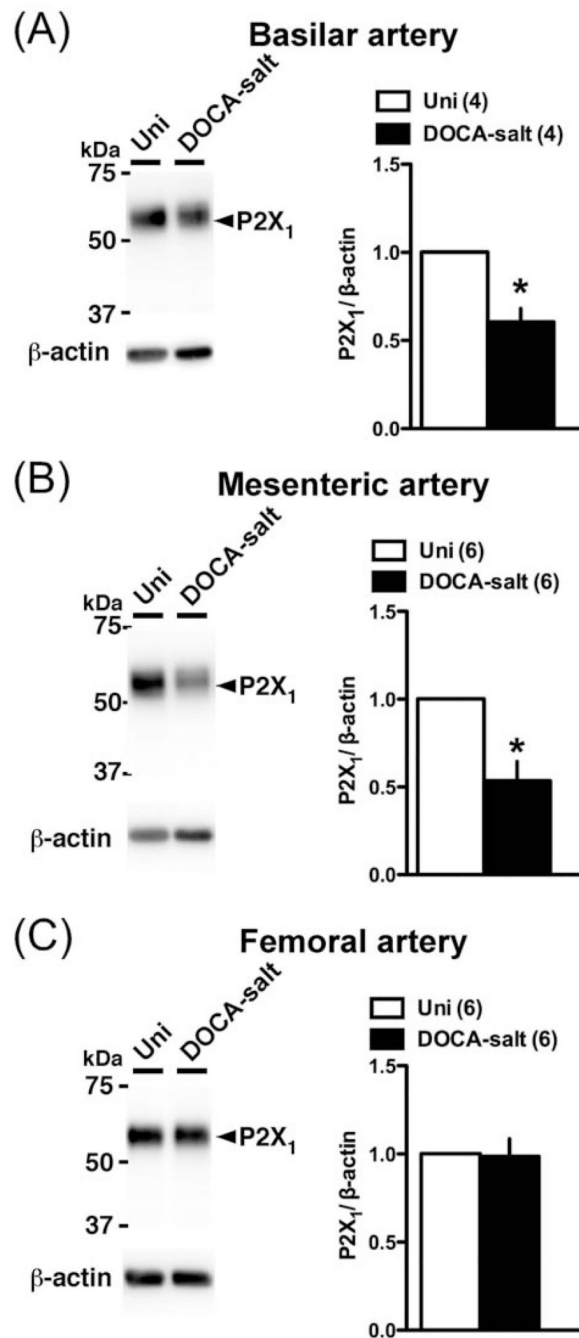


Fig. 9. Western blots for P2X₁ receptor in basilar artery (A), mesenteric artery (B), and femoral artery (C) from DOCA-salt and Uni rats. *Left:* representative Western blots for P2X₁ and β-actin. *Right:* bands were quantified as described in Materials and methods. Ratios were calculated for the optical density of each receptor over that of β-actin. Number of determinations is shown within parentheses. *P < 0.05, DOCA-salt vs. Uni group.

Table 1

Force induced by 120 mM KCl in various arterial rings from Uni and DOCA-salt rats.

Artery	Uni (mN)	DOCA-salt (mN)
Basilar	6.5 ± 0.4 (32)	5.5 ± 0.5 (34)
Thoracic aorta	25.4 ± 1.4 (25)	26.1 ± 1.3 (22)
Second-order Mesenteric	18.8 ± 0.8 (40)	17.6 ± 0.8 (37)
Femoral	19.0 ± 1.9 (28)	14.7 ± 1.4 (29)

Values are means ± SEM and represent contractile responses (in mN) to 120 mM KCl. Number of determinations is shown within parentheses.