Differential Contractile Reactivity to Nucleotides in Femoral Arteries of OLETF and LETO Rats

Takayuki Matsumoto,* Keisuke Takayanagi, Mihoka Kojima, Kumiko Taguchi, and Tsuneo Kobayashi*

Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University; Shinagawa-ku, Tokyo 142–8501, Japan.
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Extracellular nucleotides play an important role in the regulation of vascular function, and an abnormal vascular function is an important participant in the development and progression of diabetic vascular complications. The purpose of this study was to determine whether contractile responses induced by extracellular nucleotides and a dinucleotide, uridine adenosine tetraphosphate (Up4A), in femoral arteries would be altered at the chronic stage of type 2 diabetes. We determined the changes in contractile reactivity induced by ATP, uridine triphosphate (UTP), uridine diphosphate (UDP), and Up4A in the femoral arteries of Otsuka Long–Evans Tokushima Fatty (OLETF) rats (aged male type 2 diabetic rats) and, Long–Evans Tokushima Otsuka (LETO) rats (controls for OLETF rats). ATP-induced contractions were greater in OLETF rats than in LETO rats. UTP-induced contractions were lower in OLETF rats than in LETO rats. UDP- and Up4A-induced contractions were similar between OLETF and LETO rats. The femoral artery contractile changes induced by the extracellular nucleotides and dinucleotide were similar when nitric oxide synthase was inhibited. These results suggest that the extent of femoral artery contractile reactivity to nucleotides/dinucleotides differs during long-term duration of type 2 diabetes.

Key words contraction; diabetes; femoral artery; nucleotide; uridine adenosine tetraphosphate

INTRODUCTION

Diabetes is a pivotal risk factor for the development of vascular diseases, including atherosclerosis, and peripheral arterial disease, and vascular inflammation.1–3 Diabetes-associated vascular complications are the leading cause of increased morbidity and mortality worldwide.3 Since vascular dysfunction is common during long-term diabetes, comprehensive understanding of vascular function, including reactivities to endogenous vasoactive substances in each vessel type, is needed for the prevention and treatment of diabetes-associated vascular complications.

Extracellular nucleotides are important signaling molecules in various tissues and cells,4–7 including the vascular system. Extracellular nucleotides affect various properties, including atherogenesis and vascular tone.3,8–11 Moreover, a unique dinucleotide, called uridine adenosine tetraphosphate (Up4A), was identified 15 years ago. Up4A acts as an endothelium-derived contracting factor12 that leads to alterations in vascular tone, proliferation, migration, calcification, and inflammation in vascular smooth muscle cells.13,14 The responsiveness to extracellular nucleotides, including ATP, uridine triphosphate (UTP), and uridine diphosphate (UDP), and Up4A is altered in disease states such as hypertension, obesity, and diabetes.11,15–20 However, little attention has been given to contractile reactivities to such substances in the diabetic femoral artery, which is the site of frequent peripheral arterial disease complications.21

The Otsuka Long–Evans Tokushima Fatty (OLETF) rat is a type 2 diabetes model.22,23 OLETF rats are characterized by increasing body weight just after weaning, late-onset high blood glucose beginning around 18 weeks of age, diagnosable diabetes at around 24 weeks of age, and high levels of circulating insulin at 24 weeks of age that decline at around 55 weeks of age with conversion to insulin-dependent diabetes after 40 weeks of age.22,23) We and other investigations have demonstrated alterations in vascular function in OLETF rats.24–28 For example, in OLETF rats, Up4A-induced contractions were increased in renal arteries in older rats compared with young OLETF rats.19) In the thoracic aorta, Up4A-induced contractions were lower in OLETF rats at the chronic stage compared with age-matched Long–Evans Tokushima Otsuka (LETO) rats under basal conditions, and a slight relaxation was observed in OLETF rats but not in LETO rats when the aorta was pre- contracted with phenylephrine.20) In the femoral artery, increased responsiveness to noradrenaline was observed in OLETF rats at the chronic stage compared with age-matched LETO rats.26) However, the vascular reactivity to extracellular nucleotides and Up4A in the femoral arteries of the OLETF rat at the chronic stage of diabetes has not been investigated. We hypothesized that the contractile reactivity to nucleotides (i.e., ATP, UTP, and UDP) and a dinucleotide Up4A would be altered in the femoral arteries of OLETF rats compared to age-matched control LETO rats.

MATERIALS AND METHODS

Experimental Animals and Functional Studies Male OLETF and LETO rats (4 weeks of age) were purchased from Hoshino Laboratory Animals, Inc. (Ibaraki, Japan). Age-matched LETO rats were used as controls for the aged OLETF rats. Water and food were given ad libitum until the rats were sacrificed at 59 to 60 weeks of age. This study was performed in accordance with the Guiding Principles for the Care and
Use of Laboratory Animals from the Committee for the Care and Use of Laboratory Animals of Hoshi University (Tokyo, Japan).

The rats were anesthetized with isoflurane and euthanized by exsanguination. After euthanasia, the femoral artery was rapidly isolated, cleaned, and cut into rings (2 mm length) in an oxygenated modified Krebs–Henseleit solution (KHS) consisting of 118.0 mM NaCl, 4.7 mM KCl, 1.8 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.2 mM NaH\(_2\)PO\(_4\), 25.0 mM NaHCO\(_3\), and 11.0 mM glucose. The rings were suspended and set at 0.5 g (4.9 mN) optimal resting tension in an organ bath as previously described. The concentration–response curves for ATP (10\(^{-8}\) to 10\(^{-3}\) M), UTP (10\(^{-8}\) to 10\(^{-4}\) M), UDP (10\(^{-8}\) to 10\(^{-4}\) M), and Up\(_4\)A (10\(^{-7}\) to 10\(^{-4.5}\) M) were measured in the femoral arteries. In addition, the same experiments were repeated with nitric oxide synthase (NOS) inhibition by \(N^\omega\)-nitro-L-arginine (l-NNA) (a NOS inhibitor, 10\(^{-4}\) M) administered 30 min before treatment with the nucleotides or Up\(_4\)A and present thereafter.

Statistical tests were performed using Student’s t-test (GraphPad Prism ver. 8.0; GraphPad Software Inc., San Diego, CA, U.S.A.). Statistical comparisons between the two groups were performed using repeated ANOVA with Bonferroni’s multiple comparisons test (GraphPad Prism). A p-value of <0.05 indicated statistical significance.

RESULTS AND DISCUSSION

At sacrifice, body weight was lower in the OLETF rats (518.6 ± 12.3 g, n = 12) than in the LETO rats (599.8 ± 15.0 g, n = 12) (p < 0.001). Blood glucose was taken from the tail vein in non-fasted OLETF and LETO rats and was measured using a glucose meter (OneTouch Ultra, LifeScan, a Johnson & Johnson Company, Milpitas, CA, U.S.A.). Results show that blood glucose level was higher in the OLETF rats (421.7 ± 32.6 mg/dL, n = 12) than in the LETO rats (109.2 ± 5.4 mg/dL, n = 12) rats (p < 0.0001). Previous studies suggested that vascular dysfunctions were seen in various arteries obtained from at the chronic stage of type 2 diabetes. However, little attention has been given to altered vascular reactivity in response to endogenous substances in femoral arteries under type 2 diabetic conditions at the chronic stage.

Functional phenotypes induced by extracellular nucleotides and dinucleotides differ according to species, vessel types, and various diseases, including diabetes and hypertension. No investigations into the contractile reactivities to these nucleotides and Up\(_4\)A in diabetic femoral arteries have been reported. Thus, this is the first study to show different responsiveness to nucleotides and dinucleotides in femoral arteries from OLETF rats at the chronic stage of diabetes (vs. age-matched control LETO rats).

In healthy vessels, vascular tone is controlled by a balance between vascular contraction, induced by ATP derived from sympathetic nerves, and relaxation, induced by purines and pyrimidines from endothelial cells, myocytes, and erythrocytes. As shown in Figs. 1A and 2A, ATP-induced contractions were greater in femoral arteries from OLETF rats than in those from LETO rats. Nitric oxide is known as an endothelium-derived relaxing factor, and it can counteract contraction induced by various ligands. In the presence of the NOS inhibitor l-NNA, femoral artery contraction was still increased in OLETF rats in response to ATP compared with contractions in the femoral arteries of LETO rats (Fig. 2B). Therefore, the difference in ATP-induced femoral artery contractions between OLETF and LETO rats was not due to NO signaling.

Extracellular UTP can induce both relaxation and contraction. In contrast to ATP-induced contractile response, UTP-induced contractions were decreased in the femoral arteries of OLETF rats compared with LETO rats both in the presence and absence of NOS inhibition (Figs. 1B, 2C, D). Therefore, the difference in UTP-induced femoral artery contractions between OLETF and LETO rats was not because of NO signaling.

UDP induces contraction in the aortas, coronary arteries, and mesenteric arteries of mice and in the intrapulmonary arteries and carotid arteries of rat. By contrast, UDP induces relaxation in the aorta and abdominal aorta of mice and the aortas of rats. In our previous study using deoxycorticosterone acetate–salt hypertensive rats (vs. control uninephrectomized rat), femoral artery contractions induced by UDP were increased in hypertensive rat. In the present study, we observed similar UDP-induced contractile responses in femoral arteries of OLETF and LETO rats in the presence or absence of l-NNA (Figs. 1C, 2E, F). These results suggest that UDP-mediated contractions in femoral arteries are not different at the chronic stage of diabetes.

Although Up\(_4\)A was initially identified as a potent endothelium-derived contracting factor, subsequent studies showed that Up\(_4\)A induces relaxation or contraction in various vessels depending on the vessel type, species, and experimental conditions. For example, in a rat deoxycorticosterone acetate–salt hypertensive model, we demonstrated that Up\(_4\)A-induced contractions were increased in femoral, basilar, and renal arteries, whereas contractions were reduced in small mesenteric arteries and unchanged in pulmonary arteries and the thoracic aorta. In the present study, similar contractile responses were induced by Up\(_4\)A in femoral arteries from OLETF and LETO rats in the presence or absence of l-NNA (Figs. 1D, 2G, H). These results suggest that Up\(_4\)A-mediated contractions in femoral arteries are not different at the chronic stage of diabetes. On the basis of our previous and present data, we found that there was a difference in vasocontraction induced by Up\(_4\)A, depending on arteries in OLETF at the chronic stage of diabetes (vs. age-matched control LETO rats). Concretely, the following were observed: 1) The Up\(_4\)A-mediated contractions were lower in aortas in the OLETF rats than those in the LETO rats. 2) These contractions were greater in renal arteries in the OLETF rats than in those in the LETO rats. 3) These contractions were similar in femoral arteries between in the OLETF and LETO rats. Although these findings are intriguing and potentially important, future studies should investigate the age-related changes, pathophysiological roles, and mechanisms underlying these alterations.
Several limitations of the present study should be mentioned. First, several responses induced by extracellular nucleotides/dinucleotides, including ATP, UTP, UDP, and UpA, are mediated by purinoceptor activation. \(^8,9,14\) ATP activates G-protein-coupled P2Y receptors (i.e., P2Y\(_{1,2,4,6,11-14}\)) and ionotropic P2X receptors (i.e., P2X\(_{1-7}\)). \(^5,8,9\) UTP is a ligand for P2Y\(_2\) and P2Y\(_4\). UDP is a ligand for P2Y\(_6\). In addition, UpA-induced responses are mediated by the activation of P1, P2Xs, P2Ys, and non-purinergic receptors. \(^14,38\) In the rat femoral artery, Kennedy et al. \(^39\) observed that ATP could act at P2 purinoceptors in the endothelium and the smooth muscle, resulting in vasodilation and vasoconstriction, respectively. Moreover, the protein expressions of P2Y\(_2\), P2Y\(_6\), and P2X\(_1\) receptors were observed in rat femoral arteries, and these expressions did not change in the arteries between deoxycorticosterone acetate–salt hypertensive rat compared with the control uninephrectomized rats. \(^7,9\) However, we are still unable to determine which receptors contribute to the differential responsiveness in the femoral arteries of OLETF rats. Currently, it is not possible to investigate single P2 receptor subtypes because of the lack of selective antagonists. Moreover, purinoceptors are located in both endothelial cells and vascular smooth muscle cells. When receptors are stimulated by nucleotides or UpA in endothelial cells, vasorelaxant and vasoconstrictor factors are released from endothelial cells and vary depending on the vessel type and pathophysiological states, including diabetes and hypertension. \(^8,9,13,14\) Indeed, an imbalance between vasorelaxant and vasocontractile actions of endothelium-derived factors was observed in superior mesenteric arteries from OLETF rats in the chronic stage of type 2 diabetes.
diabetes. In the present study, NO may not be the primary determinant for the difference of contractions in femoral arteries between OLETF and LETO rats. However, the possibilities of modulating effects of other endothelium-derived factors, including endothelium-derived hyperpolarizing factor (EDHF) and cyclooxygenase (COX)-derived prostanoids on nucleotides/dinucleotides-induced contraction, may not be ruled out because these signaling can modulate femoral arterial contractions.

In the present study, the exact mechanisms for the differences in contractions induced by ATP and UTP in femoral arteries of OLETF and LETO rats remain unclear. The modulating effect of endothelial cells may be different, and different receptors may be activated by UTP and ATP in diabetic femoral arterial smooth muscle cells. Moreover, because UpA structurally contains both purine and pyrimidine moieties, apparent no alteration of femoral arterial contraction
induced by Up₄A between OLETF and LETO rats may result from the sum of countering effects on ATP-mediated and UTP-mediated signaling. There is a need for further investigations into the relationship between receptor expression/activation in each endothelial and smooth muscle cell, local/systemic levels of nucleotides/dinucleotides, and down-stream signaling in femoral arteries in chronic diabetes.

The pathophysiological importance of our results should also be discussed. The net balance between vasorelaxant and vasocontractile activities in patients with type 2 diabetes is abnormal, and the ability to adjust the vascular tone to meet the demands for blood flow is affected.\(^{42}\) Thanning et al.\(^{43}\) found that intra-femoral artery infusion of adenosine, ATP, and UTP all resulted in decreased vasodilation in patients with type 2 diabetes compared to control subjects. The expression and distribution of the putative P2X₃ and P2Y₂ receptors were comparable between type 2 diabetic patients and control subjects,\(^{43}\) suggesting that altered receptor sensitivity may account for the abnormal vascular responses in diabetes.

ATP is released together with noradrenaline from sympathetic nerves supplying the vasculature.\(^{4}\) Moreover, ATP is released from endothelial cells stimulated by shear stress or hypoxia.\(^{49}\) In our previous study, noradrenaline-induced contractions increased in femoral arteries of aged OLETF compared with age-matched LETO rats.\(^{56}\) Therefore, sympathetic nerve stimulation might induce augmented contractions in the femoral arteries of OLETF rats because of the additive effect of ATP and noradrenaline. Although the local levels of ATP during chronic diabetes are unclear, these findings suggest that hyperreactivity to ATP and noradrenaline may contribute to a reduction in limb blood flow after sympathetic nerve stimulation in diabetes. The relationship between the activity of sympathetic nervous system and the development and progression of peripheral arterial dysfunction with diabetes is still unclear. However, reduction of sympathetic activation may be a potential therapeutic strategy for diabetes-associated peripheral arterial dysfunction. Of note, since there are many sources of nucleotides and Up₄A and the distribution and expression of their receptors are complex under pathophysiological states, further investigations are required.

In conclusion, we demonstrated differential contractile responsiveness to ATP and UTP in the femoral arteries of OLETF and LETO rats. Although the exact pathophysiological significance of nucleotides/dinucleotides signaling in chronic diabetes is unclear, our findings should stimulate further interest in purinergic signaling as a potential therapeutic target in the continuing efforts to reduce diabetes-associated vascular diseases.

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Author Contributions TM designed the study, TM, KT, MK, KT, and TK conducted the experiments, analyzed the data, and interpreted the results. TM and TK wrote the manuscript. All authors have read and approved the manuscript.

Conflict of Interest The authors declare no conflict of interest.

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