Purinoceptor-Mediated Calcium Signaling in Preglomerular Smooth Muscle Cells

Edward W. Inscho, Elizabeth A. LeBlanc, Bao Thang Pham, Steven M. White, John D. Imig

Abstract—The current studies were performed to determine the contribution of calcium mobilization and voltage-dependent calcium influx to the increase in $[Ca^{2+}]_i$ elicited by ATP and UTP. Suspensions of freshly isolated smooth muscle cells were prepared from preglomerular microvessels by enzymatic digestion and loaded with the Ca^{2+}-sensitive dye fura 2. The effect of ATP and UTP on $[Ca^{2+}]_i$ was studied on single cells with standard microscope-based fluorescence photometry techniques. Resting $[Ca^{2+}]_i$ averaged 80±3 nmol/L (n=219 single cells from 58 dispersions). ATP (100 μmol/L) increased $[Ca^{2+}]_i$ to a peak value of 845±55 nmol/L (n=70 single cells from 38 dispersions) before stabilizing at 124±81 nmol/L. Similarly, 100 μmol/L UTP (n=39 single cells from 26 dispersions) stimulated a peak increase in $[Ca^{2+}]_i$ of 1426±584 nmol/L before reaching a stable plateau of 123±10 nmol/L. The $[Ca^{2+}]_i$ response to ATP and UTP was also assessed in the absence of extracellular calcium. In these studies, exposure to 100 μmol/L ATP induced a transient peak increase in $[Ca^{2+}]_i$, with the plateau phase being totally abolished. In contrast, exposure to 100 μmol/L UTP under calcium-free conditions resulted in no detectable change in the UTP-mediated increase in $[Ca^{2+}]_i$. The role of L-type calcium channels in the response was assessed with the calcium channel antagonist diltiazem. Incubation with diltiazem (10 μmol/L) markedly reduced the response to ATP, whereas the response to UTP was only slightly reduced. These data demonstrate that both ATP and UTP directly stimulate a biphasic increase in $[Ca^{2+}]_i$ in renal microvascular smooth muscle cells. Furthermore, the data suggest that the elevation of $[Ca^{2+}]_i$ elicited by ATP is largely dependent on calcium influx through L-type calcium channels, whereas the response to UTP appears to derive primarily from mobilization of calcium from intracellular stores. **(*Hypertension*. 1999;33[part II]:195-200.)**

Key Words: calcium channels, L-type ■ calcium, cytosolic ■ diltiazem ■ renal circulation ■ purinoceptors ■ adenosine triphosphate ■ uridine triphosphate

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Previous studies have demonstrated that afferent arterioles vasoconstrict in response to P2 receptor stimulation.[1–5] The mechanism of this vasoconstriction is at least partially dependent on the activation of voltage-gated calcium channels.[1] Subsequently, it was shown that P2 receptor inactivation or blockade significantly attenuated pressure-mediated afferent arteriolar vasoconstrictor responses.[5] This observation led to the postulate that activation of P2 receptors plays an important role in mediating renal microvascular autoregulatory responses; however, the specific P2 receptor subtype involved remains to be determined.

Recently, it was reported that P2 receptor–mediated renal vasoconstriction can be elicited by several different purine- or pyrimidine-based compounds that exhibit varying selectivity for different P2 receptor subtypes.[5,6] ATP and UTP evoke strikingly different afferent arteriolar vasoconstrictor responses; thus, suggesting the involvement of P2X (ATP) and P2Y (UTP) purinoceptor subtypes.[5] P2X and P2Y purinoceptors are structurally different receptor proteins that use different signal transduction pathways[7–13]; however, the signal transduction cascade for each has not been thoroughly investigated in the renal microcirculation. Clarification of the calcium signaling pathways accessed by ATP and UTP is important to advancing our understanding of the mechanisms by which P2 receptors influence renal microvascular tone and may provide insight into the specific P2 receptor subtype or subtypes involved in the autoregulatory response.

We have recently established the methods needed for obtaining viable vascular smooth muscle cells from freshly isolated preglomerular microvascular tissue.[14] We used this preparation to determine the effect of ATP and UTP on $[Ca^{2+}]_i$. Additional studies were performed to determine the relative contributions of calcium influx and L-type calcium channel activation on the responses elicited by ATP and UTP. Finally, we evaluated the contribution of calcium mobilization from intracellular stores in the overall response to these purinoceptor agonists. The results of these studies indicate that ATP and UTP use different intracellular signaling pathways in mediating their respective renal microvascular responses.

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From the Department of Physiology, Tulane University School of Medicine, New Orleans, La.

Correspondence to Edward W. Inscho, PhD, Department of Physiology SL#39, Tulane University School of Medicine, 1430 Tulane Ave, New Orleans, LA 70112. E-mail einscho@mailhost.tcs.tulane.edu

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Methods

Tissue Preparation and Renal Microvascular Smooth Muscle Cell Isolation

All studies were performed in compliance with the guidelines and practices dictated by the Tulane University Advisory Committee for Animal Resources. Suspensions of preglomerular microvascular smooth muscle cells were prepared with a modification of the method previously described.[14] Male Sprague-Dawley CD-VAF rats (250 to 375 g; Charles River Laboratories; Wilmington, Mass) were anesthetized with pentobarbital sodium (40 mg/kg, IV), and the abdominal cavity was exposed to permit cannulation of the abdominal aorta via the superior mesenteric artery. Ligatures were placed around the abdominal aorta at sites proximal and distal to the left and right renal arteries, respectively. The kidneys were cleared of blood by perfusion of the isolated aortic segment with an ice-cold, low-calcium physiological salt solution (low-calcium PSS; pH 7.35) of the following composition (in mmol/L): 125 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 10.0 glucose, 20.0 HEPES (*N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid), 0.1 $CaCl_2$, and 6% bovine serum albumin.[14] After the kidneys were rinsed of blood, the perfusate was changed to a similar solution containing 1% Evans blue in low-calcium PSS.

The kidneys were resected from the animal and decapsulated, and the renal medullary tissue was removed. The cortical tissue was pressed through a sieve (180 micrometer mesh), and the sieve retentate was washed several times with ice-cold low-calcium PSS. The vascular tissue remaining on the sieve was transferred to an enzyme solution containing 0.075% collagenase (Boehringer Mannheim Corp), 0.02% dithiothreitol (Sigma Chemical Co), 0.2% soybean trypsin inhibitor (type 1-S, Sigma Chemical Co), and 0.1% bovine serum albumin dissolved in low-calcium PSS, and this mixture was incubated at 37°C for 30 minutes. The vascular tissue was removed from the enzyme solution and transferred to a nylon mesh (70 micrometer mesh) where it was vigorously rinsed with ice-cold low-calcium PSS. The mesh containing the retained vascular tissue was transferred to a Petri dish containing ice-cold low-calcium PSS. Segments of interlobular artery with attached afferent arterioles were collected by microdissection using a stereoscope and transferred to a 10 mL dissociation flask. The rinse solution was decanted from the selected vascular segments and replaced with an enzyme solution containing 0.075% papain (Sigma Chemical Co) and 0.02% dithiothreitol (Sigma Chemical Co) in low-calcium PSS. The tissue was incubated at 37°C for 15 minutes before being collected by centrifugation (2000*g* for 50 seconds). The tissue pellet was transferred to an enzyme solution containing 0.3% collagenase (Boehringer Mannheim Corp) and 0.2% soybean trypsin inhibitor (type 1-S, Sigma Chemical Co) in low-calcium PSS at 37°C. After a 15-minute incubation period, the mixture was gently triturated and quickly centrifuged (500*g* for 5 minutes) to collect the dispersed cells. The supernatant was discarded, and the cells were gently resuspended in 1.0 mL Dulbecco's minimum essential medium (DMEM; Sigma Chemical Company) supplemented with 20% fetal calf serum (Whittaker Bioproducts) and 100 U/mL penicillin and 200 μg/mL streptomycin (Sigma Chemical Co). Cell suspensions were stored on ice until use.

Fluorescence Measurements in Single Microvascular Smooth Muscle Cells

Experiments were performed using a standard microscope-based fluorescence spectrophotometry system (Photon Technology International) as previously described.[14] The excitation wavelengths were set at 340 and 380 nm, and the emitted light was collected at 510±20 nm (Photon Technology Intl). Measurements of fluorescence intensity were collected at 5 data points per second, and the data were collected and analyzed with the aid of the Photon Technology International software. Calibration of the fluorescence data was accomplished as previously described.[14]

Measurement of $[Ca^{2+}]_i$ in single microvascular smooth muscle cells was performed as described previously.[14] Suspensions of freshly isolated renal microvascular cells were loaded with the calcium sensitive fluorescent probe, fura 2 acetoxymethyl ester (fura 2-AM; 4.0 μmol/L; Molecular Probes). An aliquot of cell suspension was transferred to the perfusion chamber (Warner Instrument Corporation) and mounted to the stage of a Nikon Diaphot inverted microscope. The cells were continuously superfused (1.3 mL/min) with a 1.8 millimol/L calcium PSS solution (PSS) of the following composition (in mmol/L): 125 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 10.0 glucose, 20.0 HEPES, 1.8 $CaCl_2$, and 0.111 g/L bovine serum albumin. For each experiment, a single microvascular cell was isolated in the optical field by positioning the adjustable sampling window directly over the cell of interest. Neighboring cells and debris are thus excluded from the sampling field, allowing fluorescence emission to be measured only from the cell of interest. All fluorescence measurements were obtained with background subtraction, and a new coverslip of cells was used for each experiment.

Experimental Approach

The effects of ATP and UTP on $[Ca^{2+}]_i$ were determined by exposing single cells to PSS containing ATP or UTP concentrations of 1.0, 10, or 100 μmol/L. ATP- and UTP-mediated responses were evaluated by determining the average magnitude of the peak and steady-state $[Ca^{2+}]_i$ achieved in response to the agonist. Peak responses were defined as the maximum agonist-induced $[Ca^{2+}]_i$ attained during the first 100 seconds of agonist administration. Steady-state responses were obtained by calculating the average $[Ca^{2+}]_i$ over the last 50 seconds of agonist administration.

Additional studies were performed to determine the role of extracellular calcium on the increase in $[Ca^{2+}]_i$ induced by these agonists. Cells were superfused with a nominally calcium-free solution (calcium-free PSS) resembling the PSS except that no $CaCl_2$ was added. The role of L-type calcium channels in ATP- and UTP-mediated calcium responses was assessed with the use of 10 μmol/L diltiazem. Previous studies have shown that exposure of preglomerular microvascular smooth muscle cells to 90 mmol/L KCl in a nominally calcium-free solution or in the presence of 10 μmol/L diltiazem prevent the depolarization-induced increase in cytosolic calcium.[14]

Statistical Analysis

Data are presented as mean±SEM. Within-group comparisons against the resting $[Ca^{2+}]_i$ were assessed by ANOVA for repeated measures. Differences between groups were analyzed by 1-way ANOVA. Post hoc tests were performed using the Newman-Keuls multiple range test. Statistical probabilities <0.05 ($P<0.05$) were considered significantly different.

Results

A total of 219 individual cells prepared from 58 tissue dispersions were examined in the current study. The baseline $[Ca^{2+}]_i$ in cells treated with ATP (n=121 single cells) and in cells treated with UTP (n=98 single cells) was similar and averaged 83±2 and 79±2 nmol/L, respectively. There were no significant differences in resting $[Ca^{2+}]_i$ between any of the treatment groups.

The first series of experiments determined the effects of extracellular ATP and UTP on $[Ca^{2+}]_i$ in freshly isolated rat renal microvascular smooth muscle cells, and the results of those studies are presented in Figures 1 and 2. Figure 1 presents a composite trace depicting the mean change in $[Ca^{2+}]_i$ elicited by 100 μmol/L ATP (n=70 cells) in the upper panel or 100 μmol/L UTP (n=39 cells) in the lower panel. Individual traces obtained from multiple cells subjected to an identical protocol have been combined to provide an average response profile. ATP and UTP both caused a rapid increase in $[Ca^{2+}]_i$ that reached a peak followed by a gradual recovery to a steady-state $[Ca^{2+}]_i$ that is significantly greater than control. In addition, the time course and magnitude of the

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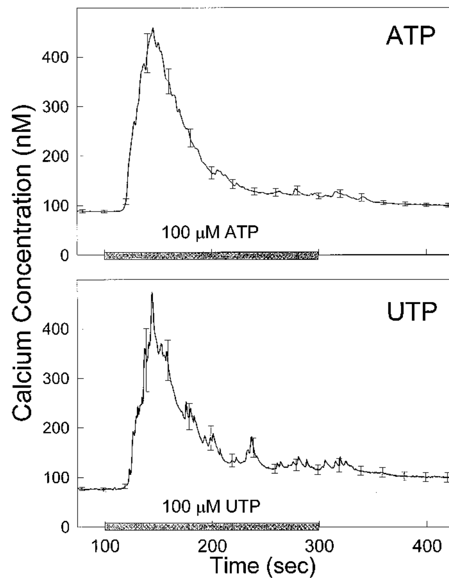

Figure 1. Response of $[Ca^{2+}]_i$ to ATP and UTP under control conditions with 1.8 mmol/L extracellular calcium. Top, The average response of 70 renal microvascular smooth muscle cells from 38 tissue dispersions to 100 μmol/L ATP. Bottom, The average response of 39 cells from 23 tissue dispersions to 100 μmol/L UTP. The period of ATP or UTP administration from 100 to 300 seconds is indicated by the gray bar along the *x* axis.

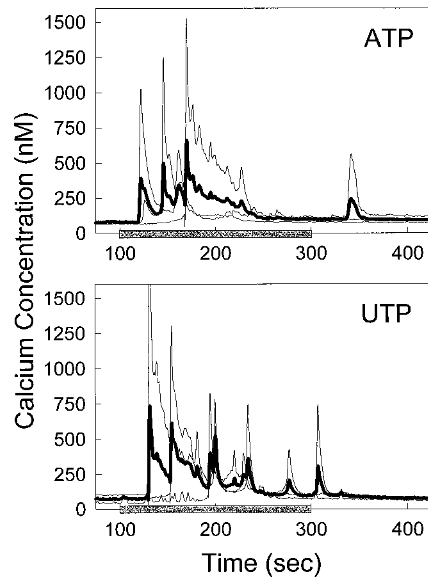

Figure 3. The effects of trace averaging on the magnitude of single peak responses obtained from individual cells stimulated with 100 μmol/L ATP (top) and 100 μmol/L UTP (bottom). Three represented calcium traces from single cells are presented by the thin lines, and the resulting calculated average trace is presented by the bold line. The period of ATP or UTP administration from 100 to 300 seconds is indicated by the gray bar along the *x* axis.

changes in $[Ca^{2+}]_i$ stimulated by ATP and UTP are similar and reversible. Removal of ATP or UTP from the bathing solution resulted in a return of the $[Ca^{2+}]_i$ to values similar to those of the controls.

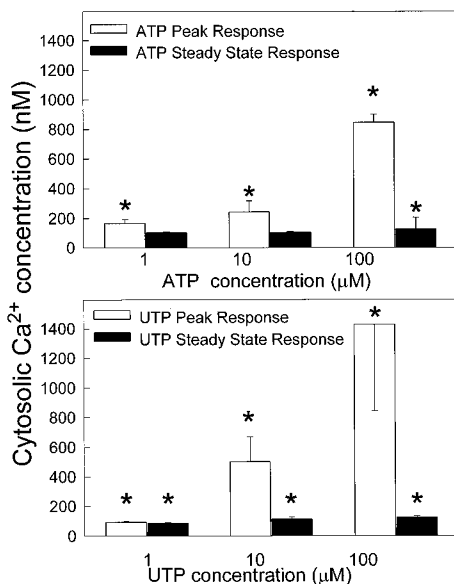

Figure 2. Effects of increasing concentrations of ATP (top) and UTP (bottom) on the $[Ca^{2+}]_i$ in microvascular smooth muscle cells. ATP and UTP were administered at concentrations of 1, 10, and 100 μmol/L. Mean peak elevations in $[Ca^{2+}]_i$ calculated by averaging the peak responses for individual cells within the first 100 seconds of agonist administration are illustrated by the open bars, and the elevations in steady-state $[Ca^{2+}]_i$ averaged over the last 50 seconds of agonist administration are depicted by the filled bars. A minimum of 11 cells from 6 tissue dispersions was used for each agonist concentration. Specific numbers for each concentration are given in the "Results." * Indicates a significant elevation of $[Ca^{2+}]_i$ over baseline.

Concentration response experiments were performed with ATP and UTP concentrations that significantly vasoconstrict rat juxtamedullary afferent arterioles.[2,5] As shown in Figure 2, ATP concentrations of 1 (n=14 cells from 7 dispersions), 10 (n=11 cells from 7 dispersions), and 100 μmol/L (n=70 cells from 38 dispersions) stimulated peak increases in $[Ca^{2+}]_i$ to 165±28, 243±73, and 845±55 nmol/L, respectively ($P<0.05$ versus the resting $[Ca^{2+}]_i$ at each ATP concentration). Sustained $[Ca^{2+}]_i$ observed with ATP concentrations of 1, 10, and 100 μmol/L averaged 102±7, 103±6, and 124±81 nmol/L, respectively, and was significantly greater at the 100 μmol/L concentration ($P<0.05$ versus the resting $[Ca^{2+}]_i$). The sustained $[Ca^{2+}]_i$ maintained during ATP administration was similar across the 3 concentrations tested.

Similar responses were observed in response to UTP administration. UTP concentrations of 1 (n=11 cells from 6 dispersions), 10 (n=12 cells from 7 dispersions), and 100 μmol/L (n=39 cells from 23 dispersions) elicited peak elevations of $[Ca^{2+}]_i$ to 93±6, 501±168, and 1426±584 nmol/L, respectively. Although the peak responses to UTP tended to be larger than the peak responses to ATP, they did not reach statistical significance. UTP also produced significant sustained elevations in $[Ca^{2+}]_i$ to 85±6, 112±15, and 123±10 nmol/L, respectively, at each concentration tested.

The composite traces shown in Figures 1, 3, 4, and 5 represent average traces generated by combining the responses of all cells tested within an experimental group. These average traces provide a more accurate view of the overall response of cells to the experimental manipulation. As described in Methods, peak responses shown in Figure 2 and described in the text were calculated by selecting the maximum agonist-induced $[Ca^{2+}]_i$ attained for each individual cell during the first 100 seconds of agonist administration and

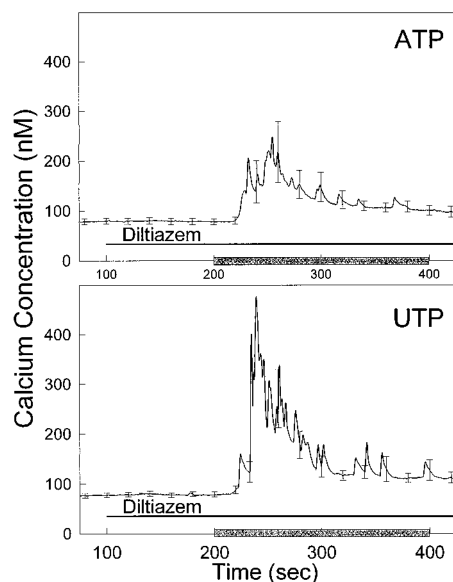

Figure 4. Responses of $[Ca^{2+}]_i$ to ATP and UTP during blockade of L-type calcium channels with diltiazem. Top, The average response of 14 renal microvascular smooth muscle cells from 5 tissue dispersions to 100 μmol/L ATP in the presence of 1.8 mmol/L extracellular calcium and 10 μmol/L diltiazem. Bottom, The average response of 13 cells from 5 tissue dispersions to 100 μmol/L UTP in the presence of 1.8 mmol/L extracellular calcium and 10 μmol/L diltiazem. The period of ATP or UTP administration from 100 to 300 seconds is indicated by the gray bar along the *x* axis. The period of exposure to diltiazem is illustrated by the thin black bar.

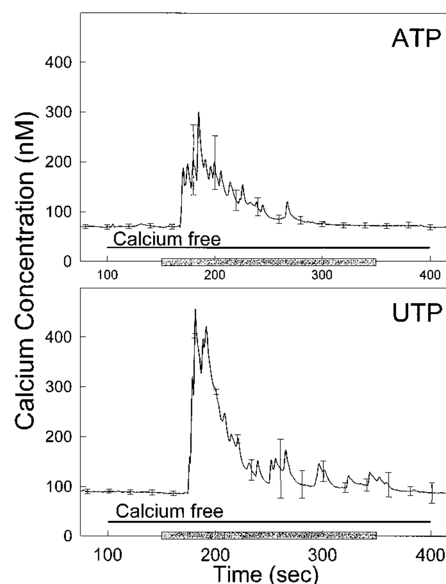

Figure 5. Responses of $[Ca^{2+}]_i$ to ATP and UTP in the absence of extracellular calcium. Top, The average response of 22 renal microvascular smooth muscle cells from 8 tissue dispersions to 100 μmol/L ATP while being bathed in calcium-free medium. Bottom, The average response of 16 cells from 7 tissue dispersions to 100 μmol/L UTP while being bathed in calcium-free medium. The period of ATP or UTP administration from 100 to 300 seconds is indicated by the gray bar along the *x* axis. The period of exposure to nominally calcium-free medium is illustrated by the thin black bar.

combining them into a group average. The time the peak increase in $[Ca^{2+}]_i$ is attained varies from 1 cell to the next but is usually achieved within 20 to 100 seconds of agonist administration. The effect of the variation in peak response time is illustrated in Figure 3. Three representative traces are given depicting the change in $[Ca^{2+}]_i$ in response to ATP (upper panel) and UTP (lower panel) and the resultant calculated trace, shown as a thick line, when the responses of the 3 individual cells are combined. The net effect of combining the individual traces is an averaged response that attenuates the magnitude of any individual peak responses. As a result, the wide variation between individual peak and steady-state $[Ca^{2+}]_i$ is smoothed into a more representative average response. It is this averaging that is responsible for the difference in the calculated peak values presented in Figure 2 and the lower but broader peak values illustrated in the composite traces shown in Figures 1, 3, 4, and 5.

Renal microvascular smooth muscle responses to ATP involve activation of voltage-gated L-type calcium channels.[1] Therefore, we determined the effect of calcium channel blockade with diltiazem on ATP- and UTP-mediated increases in $[Ca^{2+}]_i$. As shown in Figure 4, pretreatment of cells with 10 μmol/L diltiazem had no detectable effect on $[Ca^{2+}]_i$; however, it markedly reduced the overall response to ATP (Figure 4, upper panel) compared with Figure 1. Analysis of individual cell responses indicated that baseline $[Ca^{2+}]_i$ averaged 81±6 nmol/L (n=14 cells from 5 dispersions) under control conditions and 82±6 nmol/L after the addition of diltiazem to the bathing medium. Subsequent exposure to 100 μmol/L ATP increased $[Ca^{2+}]_i$ to a peak of 555±135 nmol/L before returning to a steady-state $[Ca^{2+}]_i$ of 113±11 nmol/L. Clearly from both the composite trace in Figure 4 and the individual responses, the overall increase in $[Ca^{2+}]_i$ during the period of ATP exposure is markedly reduced by diltiazem treatment. In contrast, diltiazem did not significantly alter the $[Ca^{2+}]_i$ response to UTP (Figure 4, lower panel). Compared with Figure 1, the time course and magnitude of the overall response to UTP are nearly identical. Mean data from individual cells indicate that resting $[Ca^{2+}]_i$ averaged 77±4 nmol/L (n=13 cells from 5 dispersions) during the control period and 78±5 nmol/L in the presence of diltiazem. Exposure to 100 μmol/L UTP stimulated a peak increase of $[Ca^{2+}]_i$, to 979±262 nmol/L before returning to an average steady-state $[Ca^{2+}]_i$ of 119±14 nmol/L.

We also considered the relative contribution of agonist-induced calcium mobilization from intracellular stores and calcium influx to the ATP- and UTP-induced increases in $[Ca^{2+}]_i$ in renal microvascular smooth muscle cells. These studies were performed by exposing cells to a solution containing 100 μmol/L ATP or UTP while they were being bathed in a nominally calcium-free solution. Responses obtained under calcium-free conditions were compared with control responses obtained from cells bathed in a solution containing 1.8 mmol/L Ca^{2+}. As shown in the upper panel of Figure 5, the ATP-mediated increase in $[Ca^{2+}]_i$ was markedly attenuated in cells bathed in nominally calcium-free medium. Interestingly, the overall ATP-mediated response obtained in the absence of extracellular calcium closely resembles the response obtained during blockade of L-type calcium channels (Figure 4) and is markedly reduced compared with the

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response shown in Figure 1. Analysis of the individual cell responses indicated that baseline $[Ca^{2+}]_i$ averaged 70±4 nmol/L (n=22 cells from 8 dispersions) with 1.8 mmol/L extracellular calcium and 70±4 nmol/L during incubation in the nominally calcium-free medium. Exposure to 100 μmol/L ATP under calcium-free conditions increased $[Ca^{2+}]_i$ to a peak of 689±122 nmol/L before returning to a steady-state $[Ca^{2+}]_i$ of 74±5 nmol/L. The steady-state $[Ca^{2+}]_i$ was not different from the baseline $[Ca^{2+}]_i$ in the absence of extracellular calcium.

The effect of removing extracellular calcium on the $[Ca^{2+}]_i$ response to UTP is presented in the lower panel of Figure 5. In contrast to the significant attenuation of the ATP-mediated response, the response to UTP is largely unaltered. Compared with the response shown in Figures 1 and 4, UTP elicited a temporally and quantitatively similar response despite the absence of extracellular calcium. Analysis of the individual cell responses indicated that mean resting $[Ca^{2+}]_i$ averaged 89±7 nmol/L (n=16 cells from 7 dispersions) with 1.8 mmol/L extracellular calcium and 87±7 nmol/L during incubation in the nominally calcium-free medium. Exposure to 100 μmol/L UTP under calcium-free conditions increased $[Ca^{2+}]_i$ to a peak of 863±152 nmol/L before returning to a steady-state $[Ca^{2+}]_i$ of 109±13 nmol/L. Removal of UTP from the bath resulted in a prompt return of $[Ca^{2+}]_i$ to the control values.

Control experiments were performed to verify that the concentration of diltiazem used was sufficient to block the increase in $[Ca^{2+}]_i$ produced in response to membrane depolarization with 90 mmol/L KCl. Under control conditions, 90 mmol/L KCl increased $[Ca^{2+}]_i$ from 79±24 nmol/L to 144±61 mol/L (n=25 cells from 12 dispersions). Preincubation with 10 μmol/L diltiazem nearly abolished the response to KCl such that $[Ca^{2+}]_i$ averaged 82±9 nmol/L under control conditions (n=3 cells from 1 dispersion) and 94±13 nmol/L during simultaneous exposure to 90 mmol/L KCl plus diltiazem. Additional experiments were performed to confirm that the nominally calcium-free conditions used with ATP and UTP were sufficient to prevent calcium influx in response to agonist stimulation. During superfusion of the cells with calcium-free medium, exposure to 90 mmol/L KCl actually resulted in a slight tendency for $[Ca^{2+}]_i$ to decrease from 76±13 to 71±7 nmol/L (n=7 cells from 2 dispersions). These data are in complete agreement with the findings of a previous report specifically focused on the response of these cells to depolarization.[14] Therefore, incubation of cells with diltiazem or nominally calcium-free medium was effective in eliminating depolarization-induced calcium influx.

Discussion

The current studies were performed to determine the effect of 2 different purinergic renal vasoconstrictors on the $[Ca^{2+}]_i$ in vascular smooth muscle cells freshly isolated from the preglomerular microvasculature. The results of these studies demonstrate that ATP and UTP both stimulate a rapid and biphasic increase in $[Ca^{2+}]_i$. These studies also establish that ATP and UTP elevate $[Ca^{2+}]_i$ through strikingly different mechanisms. The response to ATP involves the release of intracellular calcium and the influx of extracellular calcium. In contrast, the response to UTP appears to rely almost solely on the mobilization of calcium from intracellular stores.

Purinoceptors are a diverse group of purine and pyrimidine sensitive receptors that have been divided into major families classified as P2X and P2Y.[7,8,10,11,13] The major distinction between the P2X and P2Y subtypes relates to the structure of the receptor protein and its associated signal transduction mechanisms.[7,8,10,11,13] P2X receptors are described as having 2 membrane-spanning domains and function as ligand-gated channels. P2Y receptors have 7 membrane-spanning domains, and their function is influenced by regulatory G proteins. Both receptor families elicit cellular responses through the activation of calcium signaling pathways.[7,8,10,11,13] Stimulation of P2X receptors is believed to allow influx of extracellular Na^{+} and Ca^{2+} into the cell interior by activation of the ligand-gated channel.[15] The events that follow are more poorly understood; however, the net influx of extracellular cations could lead to membrane depolarization and the activation of voltage-sensitive calcium channels. Calcium channel activation would, in turn, lead to additional calcium influx and a further increase in the concentration of calcium in the cytosol.

There are numerous reports demonstrating the ability of extracellular ATP to elevate $[Ca^{2+}]_i$ in vascular smooth muscle cells from both renal and nonrenal tissues.[7–10,12,13,16] In the renal circulation, ATP causes renal microvascular vasoconstriction that is, in part, dependent on L-type calcium channel activation and calcium influx.[1] Like the rise in calcium shown in Figure 1, the afferent arteriolar vasoconstriction is biphasic with a rapid initial reduction in arteriolar diameter followed by a partial recovery to a new steady-state diameter.[1,2] The ATP-mediated vasoconstriction closely mimics the pattern and time course of the increase in intracellular calcium observed with cultured renal arterial vascular smooth muscle cells and freshly isolated preglomerular microvascular smooth muscle cells.[14,17] These data indicate that ATP-mediated vasoconstrictor responses are largely dependent on the influx of extracellular calcium. In the current report, calcium channel blockade and removal of calcium from the extracellular medium significantly reduced the magnitude of the ATP-mediated increase in $[Ca^{2+}]_i$. Consistent with the functional evidence generated in the intact renal microcirculation,[1] the influx of extracellular calcium is a major contributor to the ATP-mediated increase in $[Ca^{2+}]_i$ in renal microvascular smooth muscle cells and, therefore, in the ATP-mediated afferent arteriolar vasoconstriction.

In addition to stimulating calcium influx, these studies and others have revealed that ATP also stimulates the release of calcium from intracellular stores.[7–10,12,13,17] Activation of phospholipase C has also been implicated in the ATP-mediated mobilization of calcium from intracellular stores.[7–10,13] In the current report, ATP still evoked a blunted increase in $[Ca^{2+}]_i$ in the absence of extracellular calcium. A similar calcium response was obtained during calcium channel blockade. Taken together, these data suggest that the ATP-mediated increase in $[Ca^{2+}]_i$ observed during calcium channel blockade or under calcium-free conditions reflects the release of calcium from intracellular

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stores. Whether or not the involvement of calcium influx and mobilization of intracellular calcium reflects the signal transduction events initiated by a single P2 receptor subtype or multiple P2 receptor subtypes remains to be determined. Nevertheless, the renal microvascular response to P2 receptor activation includes activation of multiple signal transduction pathways involved in the regulation of $[Ca^{2+}]_i$.

We have recently reported that ATP and UTP vasoconstrict rat juxtamedullary afferent arterioles in strikingly different ways.[14] As mentioned previously, ATP stimulates a biphasic afferent arteriolar vasoconstriction.[1,2,5] In contrast, UTP elicits a monophasic response that rapidly reaches a maximum vasoconstriction that is sustained for the duration of UTP administration.[5] UTP is purported to interact primarily with G protein–regulated P2Y receptors and is reported to activate phospholipase C.[7–9,11] In the current study, UTP and ATP were found to stimulate similar increases in $[Ca^{2+}]_i$ overall; however, the mechanisms by which these agonists elevate $[Ca^{2+}]_i$ appear to be substantially different. Whereas ATP utilized both calcium influx and calcium mobilization, the response to UTP appears to arise almost exclusively from the release of calcium from intracellular stores. This conclusion is based on the observation that removal of calcium from the extracellular medium or blockade of calcium influx through L-type calcium channels had no perceptible effect on the magnitude or time course of UTP-mediated increases in $[Ca^{2+}]_i$. This suggests that binding of UTP to its receptor stimulates a signal transduction cascade designed to access stored calcium. On the basis of findings generated by other investigators, UTP-mediated activation of the phospholipase C/inositol trisphosphate/diacylglycerol pathway represents the most likely signal transduction mechanism.[9]

The marked disparity in the afferent arteriolar response to ATP and UTP[5] and the unique nature of the UTP-mediated increase in $[Ca^{2+}]_i$ strongly suggest that renal microvascular responses to ATP and UTP involve activation of 2 distinct P2 receptor subtypes. In addition, because ATP is capable of binding to both P2X and P2Y receptors, it is possible that the overall increase in intracellular calcium obtained with ATP represents the combined response from ATP binding to both P2X and P2Y receptors. For example, the calcium influx–dependent component of the ATP-mediated response could arise from activation of ligand-gated P2X receptor cation channels with the subsequent membrane depolarization and opening of L-type calcium channels. The calcium mobilization component could result from the binding of ATP to a UTP-sensitive P2Y receptor, thus leading to inositol trisphosphate 3–dependent calcium release from intracellular stores.

In summary, ATP and UTP induce a biphasic increase $[Ca^{2+}]_i$ in microvascular smooth muscle cells harvested from freshly isolated preglomerular vascular segments. Although the time course and magnitude of the overall increase in $[Ca^{2+}]_i$ are similar, the mechanisms by which they are accomplished are agonist specific. The response to ATP involves calcium mobilization from intracellular stores and calcium influx through L-type calcium channels, whereas the response to UTP develops almost solely through the release of calcium from intracellular stores.

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Edward W. Inscho, Elizabeth A. LeBlanc, Bao Thang Pham, Steven M. White and John D. Imig

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