Impaired Ca\textsuperscript{2+} Signaling Attenuates P2X Receptor–Mediated Vasoconstriction of Afferent Arterioles in Angiotensin II Hypertension

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Abstract—This study tested the hypothesis that afferent arteriolar responses to purinoceptor activation are attenuated, and Ca\textsuperscript{2+} signaling mechanisms are responsible for the blunted preglomerular vascular reactivity in angiotensin II (Ang II) hypertension. Experiments determined the effects of ATP, the P2X\textsubscript{1} agonist \(\beta,\gamma\)-methylene ATP or the P2Y agonist UTP on arteriolar diameter using the juxtamedullary nephron technique and on renal myocyte intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) using single cell fluorescence microscopy. Six or 13 days of Ang II infusion significantly attenuated the vasoconstrictor responses to ATP and \(\beta,\gamma\)-methylene ATP (\(P<0.05\)). During exposure to ATP (1, 10, and 100 \(\mu\text{mol/L}\)), afferent diameter declined by 17±2%, 29±3%, and 30±2% in normal control rats and 8±3%, 7±3%, and 22±3% in kidneys of Ang II–infused rats (13 days). Renal myocyte intracellular calcium responses to ATP or \(\beta,\gamma\)-methylene ATP were also decreased in Ang II hypertensive rats. In myocytes of control rats, peak increases in [Ca\textsuperscript{2+}]\textsubscript{i}, averaged 107±21, 170±38, and 478±79 nmol/L at ATP concentrations of 1, 10, and 100 \(\mu\text{mol/L}\), respectively. Ang II infusion for 13 days decreased the peak responses to ATP (1, 10, and 100 \(\mu\text{mol/L}\)) to 65±13, 102±20, and 367±73 nmol/L, respectively. The peak increases in [Ca\textsuperscript{2+}]\textsubscript{i}, in response to \(\beta,\gamma\)-methylene ATP were also reduced in Ang II hypertensive rats. However, angiotensin hypertension did not change the UTP-mediated vasoconstrictor responses or the myocyte calcium responses to UTP. These results indicate that the impaired autoregulatory response observed in Ang II–dependent hypertension can be attributed to impairment of P2X\textsubscript{1} receptor–mediated signal transduction. (Hypertension. 2005;46:562-568.)

Key Words: autoregulation ■ receptors, purinergic ■ calcium ■ microcirculation ■ renal circulation

The transmission of elevated blood pressure to the glomerulus and pressure-induced glomerular injury play central roles in the pathogenesis of kidney disease and its progression to end-stage renal failure. Appropriate adjustments in preglomerular resistance control glomerular filtration pressure and glomerular filtration rate. Although autoregulatory behavior is maintained or exaggerated in certain types of animal hypertension models such as spontaneously hypertensive rats, autoregulatory capability becomes impaired in models of angiotensin II (Ang II)–dependent hypertension.\textsuperscript{1–5} Impairment of afferent arteriolar autoregulatory behavior appears to involve a change in vascular smooth muscle calcium signaling.\textsuperscript{6–9} Elevation of cytosolic calcium concentration represents an important component in the afferent arteriolar response to perfusion pressure changes, and pressure-mediated vasoconstriction of afferent arterioles relies heavily on the influx of extracellular calcium through voltage-gated calcium channels.\textsuperscript{6–8}

Previous studies suggest that locally released ATP is the chemical mediator of autoregulatory responses through activation of preglomerular P2X\textsubscript{1} receptors.\textsuperscript{10–13} Numerous studies demonstrate that afferent arterioles are highly responsive to P2 receptor stimulation. ATP and other P2 agonists produce a rapid and sustained vasoconstriction of isolated rabbit afferent arterioles and rat juxtamedullary afferent arterioles.\textsuperscript{11–19} ATP induces vasoconstriction by activating P2 receptors on preglomerular microvascular smooth muscle cells.\textsuperscript{19,20} This vasoconstriction involves activation of P2X and P2Y receptors. It has been established that purinoreceptors, especially P2X\textsubscript{1} receptors, play a critical role in mediating pressure-dependent autoregulatory adjustments in afferent arteriolar diameter.\textsuperscript{21} Ablation of the P2X\textsubscript{1} receptor in genetargeted knockout mice selectively eliminated afferent arteriolar vasoconstrictor responses to the P2X\textsubscript{1} agonist \(\alpha,\beta\)-methylene ATP and markedly blunted pressure-mediated vasoconstrictor responses while retaining vasoconstrictor responses induced by A\textsubscript{1} adenosine receptor activation.\textsuperscript{21} ATP–mediated afferent arteriolar vasoconstriction is largely dependent on the influx of extracellular Ca\textsuperscript{2+}, and the sustained
vasoconstriction is maintained by Ca\(^{2+}\) influx through voltage-dependent L-type Ca\(^{2+}\) channels.\(^{15,22}\) In hypertension, autoregulatory behavior is impaired, suggesting compromised purinoceptor signaling. Therefore, the current experiments were performed to determine the involvement of the purinoceptor pathways to the blunted autoregulatory response and purinoceptor Ca\(^{2+}\) signaling mechanisms responsible for attenuated afferent arteriolar responsiveness in Ang II hypertension.

**Methods**

**Animals**
Experiments were performed on male Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC) weighing 225 to 250 g. Ang II was infused at a continuous rate by an osmotic minipump (65 ng/min) as described previously.\(^3\) All protocols were approved by the institutional animal welfare committee of the Medical College of Georgia.

**Renal Microvascular Responses**
Video microscopy experiments were conducted in vitro using the blood-perfused juxtaglomerular neprhon technique as described previously.\(^3\) Arteriolar diameters were recorded at 12-second intervals. The sustained afferent arteriolar diameter was calculated from the average of measurements made during the final 2 minutes of each treatment period. After a 25-minute equilibration period, baseline afferent arteriolar diameters were measured while perfusion pressure was set at 100 mm Hg. Then, the afferent arteriole was exposed to increasing concentrations of ATP, \(\beta\),\(\gamma\)-methylene ATP, or UTP, and diameter changes were monitored for 5 minutes at each concentration. For the renal autoregulatory experiments, afferent arteriolar diameters were measured at perfusion pressures of 100 and 160 mm Hg and again at 100 mm Hg in successive 5-minute periods.

**Renal Microvascular Smooth Muscle Cell Isolation**
Renal microvessels were isolated according to a method described previously.\(^{23}\) Briefly, rats were anesthetized with an injection of pentobarbital sodium. Kidneys were infused with a physiological salt solution (PSS), and the renal microvessels, consisting of interlobular arteries and afferent arterioles, were separated from the rest of the cortex under a stereomicroscope. Microvessels were dissected away from arterial segments arising from the inner cortical nephrons. In this regard, the cells used for calcium signaling studies were isolated from vascular segments similar to those used for the afferent arteriolar constriction and autoregulatory experiments. Renal microvessels were transferred to a digestion solution containing 0.4% albumin, 0.15% papain, and 0.05% dithiothreitol (Sigma) in low-calcium PSS at 37°C. After a 30-minute incubation period, the mixture was gently triturated and quickly centrifuged (500g for 5 minutes) to collect the dispersed cells. Cells were gently resuspended in 1 mL Dulbecco’s minimum essential medium (Sigma) and loaded with the calcium-sensitive fluorescent probe fura 2 acetoxymethyl ester (fura 2-AM; 4.0 \(\mu\)mol/L; Molecular Probes). An aliquot of cell suspension was transferred to the perfusion chamber and mounted to the stage of a Nikon inverted microscope.

Measurement of \([Ca^{2+}]_{i}\) in single microvascular smooth muscle cells was performed as described previously.\(^{23}\) The effects of purinoceptor agonists on \([Ca^{2+}]_{i}\) were determined by exposing single cells to PSS containing ATP, \(\beta\),\(\gamma\)-methylene ATP, or UTP at different concentrations. \([Ca^{2+}]_{i}\) responses were evaluated by determining the average magnitude of the peak \([Ca^{2+}]_{i}\) achieved. Peak responses were defined as the maximum agonist-induced \([Ca^{2+}]_{i}\), attained during the 200 seconds of agonist administration.

**Expression and Localization of P2X\(_1\) Receptors in Kidney**
Immunohistochemistry was performed as described previously.\(^{24}\) The formalin-fixed and paraffin-embedded cross-sections of the kidney were subjected to immunostaining assay using antibodies against rat P2X\(_1\) receptors (Alomone Laboratories).

Homogenates were prepared from the kidneys of sham-treated or Ang II–infused rats. Samples were separated by electrophoresis, transferred to a nitrocellulose membrane incubated with a primary P2X\(_1\), polyclonal antibody (1:500), followed by a secondary antibody (Santa Cruz Biotechnology), and developed using enhanced chemiluminescence as described previously.\(^{24}\)

**Data Analysis**
Statistical comparisons within each series were made using a 1-way ANOVA for repeated measurements combined with the Newman–Keuls multiple-range test. Within-group comparisons of peak \([Ca^{2+}]_{i}\), with baseline \([Ca^{2+}]_{i}\), were analyzed using ANOVA for repeated measures. The protein data were analyzed by unpaired \(t\) test. A \(P\) value of \(P<0.05\) was considered statistically significant.

**Results**
**Effect of Ang II Hypertension on Pressure-Mediated Autoregulatory Response**
Consistent with previous studies,\(^5\) chronic Ang II infusion for 6 or 14 days significantly increased systolic blood pressure (6 days 199±4 mm Hg; 13 days 214±6 mm Hg) compared with the sham-control animals (115±5 mm Hg). Afferent arteriolar diameter averaged 15.6±0.6 \(\mu\)m in sham-treated animals, at 100 mm Hg, and decreased by 19±3% (\(P<0.05\) versus control) when renal perfusion pressure (RPP) was increased to 160 mm Hg (Figure 1). Returning RPP to 100 mm Hg resulted in a complete recovery to 14.8±0.4 \(\mu\)m. In contrast to the sham animals, increasing RPP to 160 mm Hg in rats infused with Ang II for 6 and 13 days reduced afferent diameter by 8±2% and 9±2%, respectively (\(P<0.05\) versus sham group; Figure 1). The magnitude of the pressure-mediated vasoconstriction is significantly smaller in angiotensin-dependent hypertension.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Afferent arteriolar response to increasing RPP in kidneys of normotensive and Ang II hypertensive rats. Values are means±SE. *\(P<0.05\), significant difference from 100 mm Hg; #\(P<0.05\), significant difference from normotensive controls; \(n=20\) to 23 afferent arterioles studied.
Effect of Ang II Hypertension on Afferent Arteriolar Response to ATP

The response of afferent arterioles to ATP stimulation is presented in Figure 2. In sham-treated animals, afferent arteriolar diameter declined by 8±2%, 17±3%, 29±3%, and 30±2% for ATP concentrations of 0.1, 1, 10, and 100 μmol/L, respectively. Ang II infusion for 6 or 13 days significantly blunted the vasoconstrictor responses to ATP. On day 6 of Ang II infusion, ATP (0.1, 1, 10, and 100 μmol/L) induced a vasoconstriction of 3±3%, 2±4%, 8±3%, and 13±7%, respectively (Figure 2). Similar results were observed in kidneys from 13-day hypertensive rats. These data demonstrate that afferent arteriolar responses to ATP stimulation are attenuated in angiotensin-dependent hypertension.

Effect of Ang II Hypertension on Afferent Arteriolar Response to P2X<sub>1</sub> Receptor Stimulation

In separate experiments, we determined the response of afferent arteriolar diameter to a selective P2X<sub>1</sub> agonist, β,γ-methylene ATP. In sham-treated animals, β,γ-methylene ATP (0.1, 1, 10, and 100 μmol/L) significantly reduced afferent diameter by 6±1%, 14±3%, 19±3%, and 22±3%, respectively (Figure 3). The afferent diameter response to P2X<sub>1</sub> agonist is markedly reduced in Ang II–infused hypertensive rats. The vasoconstrictor responses to β,γ-methylene ATP (0.1, 1, 10, and 100 μmol/L) were 1±3%, 6±3%, 2±3%, and 4±4% on day 6 and 1±2%, 3±6%, 7±6%, and 10±5% on day 13 of Ang II infusion, respectively (Figure 3).

Effect of Ang II Hypertension on Afferent Arteriolar Response to P2Y Receptor Stimulation

The afferent arteriolar response to the P2Y agonist UTP was investigated to determine whether P2Y receptors are involved in the attenuated vasoconstriction to ATP. In sham-treated rats, afferent diameter decreased by 19±6%, 36±7%, and 71±4% for UTP concentrations of 1, 10, and 100 μmol/L, respectively (Figure 4). Ang II infusion did not significantly alter the vasoconstrictor response to UTP. In response to UTP (1, 10, and 100 μmol/L), afferent arteriolar diameter reduced by 4±2%, 20±4%, and 66±5% in kidneys from rats infused with Ang II for 6 days, respectively. Similar results were obtained in the afferent arterioles of rats infused with Ang II for 13 days (Figure 4).

Effect of Ang II Hypertension on Afferent Arteriolar Response to A<sub>1</sub> Receptor Stimulation

Activation of A<sub>1</sub> receptors is postulated to mediate tubuloglomerular feedback responses. Accordingly, experiments were performed to compare the afferent arteriolar responses to adenosine in sham-treated and Ang II–infused animals. Superfusion with 1 and 10 μmol/L adenosine reduced afferent arteriolar diameter from 14.9±0.8 to 12.8±0.8 μm and 13.0±1.1 μm (n=6), respectively. Chronic Ang II infusion did not significantly alter the afferent arteriolar diameter response to adenosine. In response to adenosine (1 and 10 μmol/L), afferent arteriolar diameter decreased from 14.1±0.8 to 13.4±0.7 μm and 13.5±0.9 μm (n=5) in rats infused with Ang II for 13 days.

Renal Microvascular Smooth Muscle Cell [Ca<sup>2+</sup>]<sub>i</sub> Response to P2X<sub>1</sub> Agonist

To test the hypothesis that purinoceptor Ca<sup>2+</sup> signaling mechanisms are responsible for impaired afferent arteriolar responsiveness to purinoceptor activation, we investigated the
[Ca\(^{2+}\)]\(_i\), response to \(\beta,\gamma\)-methylene ATP in the microvascular smooth muscle cells isolated from normal control and Ang II–infused hypertensive rats. Figure 5A shows typical traces depicting the [Ca\(^{2+}\)]\(_i\), changes evoked by \(\beta,\gamma\)–methylene ATP in sham- and Ang II–treated animals. Exposure of renal myocytes to 10 and 100 \(\mu\)mol/L \(\beta,\gamma\)-methylene ATP evoked a biphasic increase in [Ca\(^{2+}\)]\(_i\), that typically included a rapid peak response followed by a gradual return to steady-state level similar to baseline. Ang II infusion (13 days) significantly decreased the renal myocyte [Ca\(^{2+}\)]\(_i\) by 127±28, 164±38, and 449±77 nmol/L, respectively, in sham animals (Figure 6A). On day 13 of Ang II infusion, the peak [Ca\(^{2+}\)]\(_i\) responses to 10 and 100 \(\mu\)mol/L \(\beta,\gamma\)-methylene ATP averaged 45±13 and 77±16 nmol/L in Ang II–treated animals compared with 137±38 and 175±75 nmol/L in sham rats (Figure 5B).

Renal Microvascular Smooth Muscle Cell [Ca\(^{2+}\)]\(_i\) Response to ATP and UTP

Consistent with previous data,\(^{22}\) exposure to ATP evoked a rapid response, followed by a steady-state plateau. ATP (1, 10, and 100 \(\mu\)mol/L) increased renal myocyte [Ca\(^{2+}\)]\(_i\), by 127±28, 164±38, and 449±77 nmol/L, respectively, in sham animals (Figure 6A). On day 13 of Ang II infusion, the peak renal myocyte [Ca\(^{2+}\)]\(_i\) responses to 1, 10, and 100 \(\mu\)mol/L ATP were 63±12, 128±36, and 367±73 nmol/L (Figure 6A). These data suggest that Ang II hypertension attenuates the initial [Ca\(^{2+}\)]\(_i\) response to P2 receptor activation.

In sham-treated animals, UTP (10 and 100 \(\mu\)mol/L) caused an increase in [Ca\(^{2+}\)]\(_i\), that rapidly peaked (253±68 and 529±93 nmol/L) and gradually declined to a steady-state concentration (29±7 and 41±7 nmol/L) greater than baseline. Ang II infusion (13 days) did not significantly alter the renal myocyte [Ca\(^{2+}\)]\(_i\), responses to ATP, and the peak responses to UTP (10 and 100 \(\mu\)mol/L) averaged 215±65 and 584±150 nmol/L, respectively (Figure 6B).

Expression and Localization of P2X\(_1\) in Kidney

Immunohistochemical staining for P2X\(_1\) purinoceptors show that P2X\(_1\) purinoceptors were found in the intrarenal vasculature from the interlobular artery to the afferent arterioles but not the glomeruli (Figure 7A). Figure 7B presents a representative Western blot demonstrating that P2X\(_1\) receptor expression is uniquely microvascular. Dose-dependent increases in P2X\(_1\) protein detection are evident for vas deferens and renal microvascular tissues, whereas no detectable band was noted for whole homogenates of renal cortex and medulla. Figure 7C presents representative Western blots of P2X\(_1\) receptor protein levels were not changed in Ang II hypertensive rats 6 days 1.6±0.2 versus 13 days 1.7±0.2 versus normal control 1.8±0.4 density units; n=6).
Autoregulatory control of preglomerular resistance is an essential component of normal renal hemodynamics. For certain models of hypertension, autoregulatory efficiency is compromised. Although the existence of autoregulatory behavior is recognized, the mechanisms responsible for effecting pressure-mediated preglomerular vasoconstriction remain unclear. We postulated that locally released ATP mediates autoregulatory responses by activating P2X1 receptor. The current study was undertaken to test the hypothesis that impairment of autoregulatory behavior in Ang II hypertension results from reduced afferent arteriolar responsiveness to P2X1 receptor activation. Ang II hypertension exhibits attenuated autoregulatory responses as shown by reduced pressure-mediated afferent arteriolar vasoconstriction. Afferent arteriolar responses to purinoceptor stimulation were significantly attenuated in kidneys of Ang II–treated animals. Most notably, vasoconstrictor responses to P2X1 receptor activation were nearly abolished, whereas responses to adenosine and UTP were unaltered. The current studies also demonstrated decreased intracellular calcium responses to ATP and the selective P2X1 receptor agonist AT9252, whereas responses to adenosine and UTP were unaltered. The current studies also demonstrated decreased intracellular calcium responses to ATP and the selective P2X1 receptor agonist AT9252, whereas responses to adenosine and UTP were unaltered. These data suggest that the elevated blood pressure, not elevated Ang II influences, are causative in the decline in autoregulatory efficiency in the Ang II–infused hypertensive rats. Thus, based...
on these previous findings and the result of the current study, it appears that P2 purinoceptor pathway, especially P2X-receptors, is involved in the reduced autoregulatory efficiency observed in Ang II–dependent hypertension.

Preglomerular microvascular autoregulatory responses to changes in perfusion pressure are blunted in kidneys from Ang II–infused hypertensive rats. Casellas et al demonstrated that autoregulatory responses were impaired in juxtamedullary afferent arterioles and interlobular arteries and that the greatest degree of impairment was observed in close proximity to the glomerulus. Similar impairment was noted in 2K1C Goldblatt hypertensive rats. Inscho et al further demonstrated that impairment of autoregulatory responsiveness was associated specifically with the kidney, and not circulating vasoactive agents in the perfuse blood, because autoregulatory behavior could not be restored by perfusing hypertensive kidneys with blood from normotensive donors or with Tyrode’s buffer. It has been established that P2 receptors, especially P2X receptors, play a critical role in mediating pressure-dependent autoregulatory adjustments in afferent arteriolar diameter. Ablation of the P2X receptor, in gene-targeted KO mice, selectively eliminated afferent arteriolar vasoconstrictor responses to the P2X agonist α,β-methylene ATP and markedly blunted pressure-mediated vasoconstrictor responses while retaining adenosine-mediated vasoconstrictor responses induced by A1 receptor activation. Our current studies confirmed attenuated pressure-mediated autoregulatory responses in Ang II–infused hypertensive kidneys. Furthermore, Ang II infusion for 6 and 13 days also significantly decreased afferent arteriolar diameter responses to ATP while retaining normal vasoconstrictor profiles to adenosine. These data suggest that impairment of P2X receptor signaling may be critically involved in the blunted autoregulatory behavior observed in hypertensive animals.

Autoregulation is accomplished through the combined influences of myogenic and tubuloglomerular feedback responses. Adenosine has been the leading candidate for tubuloglomerular feedback (TGF) responses for many years, but the debate on the active agent continues. In the current report, TGF-dependent responses were not assessed but the afferent arteriolar response to P1 and P2 receptor activation and the overall autoregulatory response to changes in perfusion pressure were determined. Clearly, the impaired autoregulatory response correlated strongly with impaired P2X-receptor-mediated vasoconstriction and elevations in intracellular calcium. Vasoconstrictor and calcium signaling responses to the endogenous ligand ATP were also significantly attenuated in kidneys from hypertensive animals. Interestingly, responses to adenosine and P2Y receptor activation were unchanged in Ang II–infused animals. Although these data do not specifically address the status of the TGF response, they do support the argument that ATP-dependent activation of P2X receptors plays a major role in autoregulatory responses and that impairment of P2X receptor signaling may result in compromised autoregulatory control.

It is well established that P2 receptor activation leads to an increase in intracellular calcium concentration, resulting in agonist-induced afferent arteriolar vasoconstriction and autoregulatory adjustments in afferent arteriolar diameter. Therefore, in the current study, renal myocyte calcium responses to ATP were also evaluated, and our results demonstrate that intracellular calcium responses to ATP were significantly attenuated in Ang II hypertension. Renal microvascular smooth muscle cell [Ca2+]i increases, the mechanisms by which they elevate [Ca2+]i are substantially different. ATP uses calcium influx and calcium mobilization, whereas the response to UTP seems to arise almost exclusively from the release of calcium from intracellular stores. In the current study, we measured the [Ca2+]i response to β,γ-methylene ATP and UTP in preglomerular myocytes from the inner cortex of normotensive and hypertensive rats. Consistent with the functional findings, the intracellular calcium response to β,γ-methylene ATP was significantly reduced in microvascular smooth muscle cells isolated from Ang II–infused hypertensive animals. However, Ang II infusion did not change the renal myocyte [Ca2+]i response to UTP. It should be noted that the preglomerular smooth muscle cells used in the current study originated from microvascular segments that were similar to those used for the autoregulatory and vasoconstriction experiments, begging the question of how our findings apply to the whole cortex. Consistent with our findings of impaired autoregulation and impaired P2 receptor–mediated vascular responses, Wang et al demonstrated that whole kidney autoregulation of renal blood flow was impaired in Ang II–infused rats. These data support our hypothesis that angiotensin hypertension attenuates preglomerular autoregulatory responses and calcium signaling and is responsible for the decreased afferent arteriolar responses to purinoceptor stimulation.

Kidneys from hypertensive rats possess impaired responsiveness to P2X receptor activation and compromised autoregulatory capability. These functional observations could be explained by reduced P2X receptor expression by preglomerular microvascular smooth muscle cells. Consistent with the observation of Chan et al, our Western blot and immunohistochemical studies revealed preferential P2X receptor protein expression by preglomerular microvessels with no protein expression noted in whole homogenate of renal cortex or medulla. Interestingly, no significant difference was detected in P2X receptor expression between microvessels collected from normotensive kidneys compared with similar tissues collected from kidneys after 6 and 13 days of Ang II infusion. These data suggest that the link of autoregulatory control and reduced responses to P2X receptor stimulation in Ang II hypertension cannot be explained by downregulation of P2X receptor expression. The studies performed examining the effect of Ang II hypertension on calcium signaling responses offer another possible explanation for reduced autoregulatory efficiency and responsiveness to P2 receptor stimulation. Intracellular calcium is a major signaling molecule in vascular smooth muscle, and calcium influx is a major component of autoregulatory responses and P2X receptor–mediated afferent arteriolar vasoconstriction. The current...
report establishes that P2X<sub>1</sub> receptor–dependent increases in preglomerular smooth muscle cell calcium are markedly attenuated in kidneys from hypertensive rats. This impaired Ca<sup>2+</sup> influx response could explain the attenuated vasoconstriction elicited by an increase in RPP or by P2X<sub>1</sub> receptor activation. This observation is consistent with the hypothesis that P2X<sub>1</sub> receptor activity is an essential first step in pressure-mediated autoregulatory adjustments inafferent arteriolar resistance.

**Perspectives**

The results of the present study demonstrate that afferent arteriolar responses to increasing perfusion pressure and purinoreceptor activation are blunted in Ang II–infused hypertensive rats. Consistent with the functional changes, [Ca<sup>2+</sup>]i responses to ATP and β<sub>γ</sub>-methylene ATP are attenuated in VSMCs isolated from hypertensive animals. This study provides compelling new evidence for P2X<sub>1</sub> receptor activation as an essential early step in mediating pressure-dependent autoregulatory adjustments in afferent arteriolar diameter. Furthermore, the current study demonstrates that the impaired autoregulatory behavior in Ang II–dependent hypertension can be attributed to attenuated P2X<sub>1</sub> receptor–dependent calcium signaling, resulting in impaired P2X<sub>1</sub> receptor–mediated afferent arteriolar vasoconstriction.

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