Acute In Vivo Analysis of ATP Release in Rat Kidneys in Response to Changes of Renal Perfusion Pressure

Oleg Palygin, PhD*; Louise C. Evans, PhD*; Allen W. Cowley, Jr, PhD; Alexander Staruschenko, PhD

Background—ATP and derivatives are recognized to be essential agents of paracrine signaling. It was reported that ATP is an important regulator of the pressure-natriuresis mechanism. Information on the sources of ATP, the mechanisms of its release, and its relationship to blood pressure has been limited by the inability to precisely measure dynamic changes in intrarenal ATP levels in vivo.

Methods and Results—Newly developed amperometric biosensors were used to assess alterations in cortical ATP concentrations in response to changes in renal perfusion pressure (RPP) in anesthetized Sprague-Dawley rats. RPP was monitored via the carotid artery; ligations around the celiac/superior mesenteric arteries and the distal aorta were used for manipulation of RPP. Biosensors were acutely implanted in the renal cortex for assessment of ATP. Rise of RPP activated diuresis/natriuresis processes, which were associated with elevated ATP. The increases in cortical ATP concentrations were in the physiological range (1–3 µmol/L) and would be capable of activating most of the purinergic receptors. There was a linear correlation with every 1-mm Hg rise in RPP resulting in a 70-nmol/L increase in ATP. Furthermore, this elevation of RPP was accompanied by a 2.5-fold increase in urinary H₂O₂.

Conclusions—Changes in RPP directly correlate with renal sodium excretion and the elevation of cortical ATP. Given the known effects of ATP on regulation of glomerular filtration and tubular transport, the data support a role for ATP release in the rapid natriuretic responses to acute increases in RPP. (J Am Heart Assoc. 2017;6:e006658. DOI: 10.1161/JAHA.117.006658.)

Key Words: ATP • hypertension • kidney • P2 receptors • pressure natriuresis • purinergic signaling • renal

The role of the purinergic system in the regulation of kidney function, however, has received increasing attention. ATP is known to contribute to the regulation of many fundamental renal processes including the myogenic response, tubuloglomerular feedback, and, importantly, sodium and water transport along all segments of the nephron. In addition to its constitutive release, ATP secretion occurs in response to physiological stimuli, as observed in response to increased luminal flow. Bell et al estimated that the ATP concentration at the basolateral surface of the macula densa in the presence of increased luminal sodium chloride approached 10 µmol/L. Even assuming rapid hydrolysis of cortical ATP, these concentrations are sufficient to promote strong physiological responses.

Given that pressure natriuresis is a mechanically initiated phenomenon, we hypothesized that ATP release with elevations of renal perfusion pressure (RPP) might be involved in its regulation. Navar and colleagues first demonstrated, using microdialysis techniques, that elevations of RPP stimulated elevations of tissue ATP concentrations. A microdialysis approach, however, can significantly underestimate tissue extracellular ATP levels, given the very rapid hydrolysis of ATP into adenosine and uric acid. The goal of this study was to apply a real-time electrochemical detection method to...
Role of ATP in Pressure

animal care and use committee.
and approval by the Medical College of Wisconsin institutional Care and Use of Laboratory Animals, following protocol review guidelines of the National Institutes of Health Guide for the present studies, adhering to the animal use and welfare

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Clinical Perspective

What Is New?
• The current report represents an important technical advance in which we used biosensor amperometry to measure in vivo changes in cortical ATP in real time.
• The data implicate ATP as a key regulator of the pressure-natriuresis mechanism.

What Are the Clinical Implications?
• Our results show that biosensors can be used for measuring analytes, such as purines or reactive oxygen species, in unprocessed whole blood or spot urine samples.
• Levels of cortical ATP correlate directly with arterial pressure and urinary sodium excretion.

Methods

Experimental Animals and Surgical Preparation

Male Sprague–Dawley rats obtained from Envigo were used in the present studies, adhering to the animal use and welfare guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, following protocol review and approval by the Medical College of Wisconsin institutional animal care and use committee.

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Null and ATP biosensors were obtained from Sarissa Biomedical Inc and were used previously to assess extracellular ATP and H₂O₂ concentrations in isolated perfused kidneys. A novel series of these biosensors enabled application of these techniques to in vivo blood-perfused kidneys in the present studies. Specifically, the new Sarissagold series biosensors utilize gold electrodes coated with Ruthenium Purple as an electron mediator. This gives a greater specificity against interference from 5HT, ascorbic acid, urate, and acetaminophen and provides high stability and sensitivity in physiological fluid samples, such as blood.

The operating potential of Sarissagold biosensors is in the range of 0 to −50 mV with respect to the Ag/AgCl reference electrode. As noted in the studies presented, the biosensors detect a reduction current, rather than the oxidation current like the Sarissaprobe biosensors, whereby the signal generated is inversely proportional to the concentration of ATP. Calibration of the sensors was performed in a small silicon-bottom coated Petri dish with 3 mL of calibration buffer containing 100 mmol/L NaCl, 10 mmol/L NaPi, 1 mmol/L MgCl₂, and 2 mmol/L glycerol, pH 7.4 (Figure 2). Before calibration, the sensors were polarized for 30 seconds at +600 mV. Linear increases in the biosensor responses were observed in response to ATP over the range of 0.2 to 10 μmol/L, as measured by amperometry with a dual-channel DY2021 potentiostat and a recording system. The null biosensor showed no sensitivity to ATP application. Hydrogen peroxide (H₂O₂) application produced similar responses in both sensors and was eliminated in the calculation procedure by signal differentiation between the ATP and null responses, as described previously. The addition of the scavenger enzyme catalase (2 mg/mL; ≥10 000 U/mg protein) or the ATP-hydrolyzing enzyme apyrase (1 mg/mL; ≥100 U/mg protein) into the calibration chamber rapidly blocked H₂O₂ and ATP signals, respectively. After calibration, both sensors were implanted in the left kidney using micromanipulators. The tips of the sensors (500 μm long and 75 μm in diameter) were placed in the renal cortex. The reference electrode was placed on the kidney surface and connected to the potentiostat ground to compensate for the electrostatic noise, providing low-noise amperometric recordings. All experiments were performed on a high-performance vibration isolation

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table contained in a Faraday cage to reduce electrical and mechanical noise. All recorded signals were filtered with a low-pass filter with a cutoff frequency of 0.1 Hz and digitized at a sampling rate 5 Hz.

Experimental Protocol
Baseline measurements of MAP were obtained over a 15-minute period during which urine was collected bilaterally from both ureter catheters and the resting concentration of cortical ATP was monitored by the implanted biosensors. MAP was then abruptly increased by ≈25 to 30 mm Hg by occlusion of the superior mesenteric and celiac arteries and aorta distal to both kidneys, and changes in cortical ATP concentration were monitored, urine was collected, and pressure was recorded for another 15 minutes before releasing the arterial ties. This process was repeated, resulting in 3 baseline pressure periods and 2 pressure-step periods over the duration of the study, each of which lasted 15 minutes. In some experiments, the higher pressure was kept for a longer time to assess probe desensitization.
Urinary Analysis

Urine volumes collected during the 15 minute intervals were determined gravimetrically, assuming a density equal to water. Urine sodium and potassium concentrations were measured by flame photometry and used for the calculation of urinary sodium and urinary potassium, as described previously. In an independent set of experiments, the amount of ATP and H₂O₂ was determined in the urine collected during control or pressure-step conditions. Immediately after each 15-minute collection interval, biosensors were inserted in a 300-μL urine sample, and the corresponding enzyme was added to estimate urinary ATP and H₂O₂ concentration.

Statistical Analyses

The sensitivity of the biosensors was estimated by a linear fit with least squares estimation (OriginPro 9.0; OriginLab Corp) of the recorded calibration points, as described earlier. Experimental data points were recalculated using this calibration curve for each pair of biosensors, and variable interdependence was assessed using the Pearson correlation coefficient. Correlation coefficients with magnitude ≥0.7 were considered highly correlated. The data were analyzed using 1-way ANOVA with normality (Shapiro–Wilk), and equal variance tests were Holm–Sidak or Bonferroni methods of pairwise multiple comparisons were applied. A repeated measures ANOVA was used to assess between-group differences in dependent variables over time, and independent pairwise multiple comparison procedures (Holm–Sidak method) were used to determine differences in the means of treatment groups. All summarized data are reported as mean±SEM. Differences were considered statistically significant at P<0.05.

Results

Application of Biosensors to Measure ATP In Situ

As described in Methods, rats were surgically prepared for the assessment of cortical ATP concentrations in response to step changes in arterial pressure (Figure 1). To determine endogenous concentrations of ATP in blood-perfused kidneys, the ATP and null biosensors were inserted into the renal cortex. Before insertion into the renal cortex, the biosensors were calibrated in a separate chamber and subjected to various concentrations (0.33–2.35 μmol/L) of exogenous ATP. As shown in Figure 2B, linear relationships between the amperometric signal (pA) and ATP concentrations (μM) were observed. The sensors displayed a response at concentrations as low as 200 nmol/L and as high as 10 μmol/L, enabling experimental quantification of kidney ATP levels within this range. The ATP-hydrolyzing enzyme

Figure 2. Calibration and analysis of the sensitivity of ATP biosensors. A, Schematic of the protocols used for sensors rehydration and calibration. B, Calibration of ATP sensors based on known ATP concentrations (Pearson r=0.99; ANOVA P=0.013). The addition of ATP (0.33, 1, 1.65, and 2.35 μmol/L) produces an amperometric current on the sensor that was used for preparing calibration curve (on the right). The ATP-hydrolyzing enzyme apyrase was added at the end to confirm the specificity of the sensors.
apyrase was added to each sensor at the end of each calibration process to confirm the specificity of the sensors.28 The specificity of the sensors for ATP was established previously by their insensitivity to the purinergic molecules ADP, UTP, UDP, and adenosine.26,30

Rapid Changes of Cortical ATP Concentrations Following Step Changes of RPP

The rapid response of the sensors to ATP, within seconds (see Figure 2B), enabled the accurate real-time measurement of cortical ATP concentrations in response to acute changes in RPP. The dynamic relationship between RPP and cortical ATP levels is shown in Figure 3A. Following the first step increase of RPP, a substantial increase in cortical ATP concentration was observed, as shown by the downward deflection of the amperage signal. After an initial delay, ATP levels in the kidney cortex were significantly increased, within 5 to 10 minutes. On release of the arterial ligations, both the reduction of ATP concentration and the normalization of arterial pressure were nearly instantaneous. The second pressure step induced a more rapid onset and progressive increase in cortical ATP concentrations throughout the 15-minute period. Again, the release of the ligations and the normalization of arterial pressure resulted in an immediate restoration of ATP to control levels.

Figure 3B summarizes changes in the cortical ATP concentrations in response to modulation of RPP. Notably, we observed a linear correlation between arterial pressure and cortical ATP concentration (N=9 rats; Pearson r=0.74; ANOVA P<0.05), with each 1-mm Hg change in MAP resulting in ≈70-nmol/L change in ATP concentration. This relationship was not different when MAP was either increased (63.6±14.8 nmol/L per 1 mm Hg; n=9) or decreased (76.3±16.2 nmol/L per 1 mm Hg; n=9). Clamping arterial pressure for longer periods did not result in desensitization. Elevations of RPP up to 40 minutes produced a constant elevation of cortical ATP concentration (Figure 4), which also rapidly decreased after the ligations were removed and MAP returned to the basal level.

Relationship of RPP to Sodium, Potassium, and Water Excretion

Figure 5 summarizes the changes in MAP (Figure 5A), urinary flow (Figure 5B), sodium (Figure 5C), and potassium (Figure 5D) excretion in response to step changes in RPP. The first pressure step caused a 29±2-mm Hg increase in MAP from an average control of 98±6 mm Hg to 128±7 mm Hg (Figure 5A). Urine flow increased nearly 5-fold during the first high-pressure period compared with the first control period (27±11 compared with 145±28 µL/min; Figure 5B). As expected, changes in RPP were associated with a significant increase in urinary sodium excretion (from 2.49±0.98 to 18.66±3.76 µmol/min; Figure 5C) and potassium excretion (from 1.24±0.33 to 3.01±0.50 µmol/min; Figure 5D). Following release of the arterial ligations, RPP returned to the control level, averaging 105±7 mm Hg (Figure 5A, control 2), which was accompanied by the return of urinary flow and sodium and potassium excretion to levels comparable to those recorded during the first control period (urinary flow 30±6 µL/min, urinary sodium excretion 3.75±0.92 µmol/min, and urinary potassium 2.35±0.48 µmol/min). Notably, despite a comparable increase in RPP (26±6 mm Hg), the

Figure 3. Renal perfusion pressure induced changes of ATP concentration in the kidney cortex. A, Experimental protocol, 5×15-min manipulation periods. Changes in mean arterial pressure (MAP) and ATP levels during pressure manipulation by ligation occlusion/release (note the direction of the signal shown in the left-hand panel). B, Summary graphs represent changes in ATP concentration from the baseline (control) value detected by microenzymatic biosensors in kidney cortex (N=9 rats).
Role of ATP in Pressure–Natriuresis  Palygin et al

Figure 4. Renal perfusion pressure (RPP) promotes the steady release of ATP in the kidney. Prolonged elevation of mean arterial pressure (MAP) triggers a continuous release of cortical ATP.

diuretic (62±17 µL/min) and natriuretic (8.78±2.33 µmol/min) responses to the second pressure step were attenuated compared with the first step, indicating volume contraction. Again, however, release of the arterial ligations caused the normalization of urinary parameters (urinary flow was 12±5 µL/min, urinary sodium and potassium excretions were 1.28±0.65 µmol/min and 0.94±0.32 µmol/min, respectively) and blood pressure (109±7 mm Hg).

H2O2 Concentrations in the Urine

The same amperometry approach was also applied to analyze changes of H2O2 levels in the urine samples collected during an independent set of experiments with step changes in RPP. Urine was collected, and urinary concentrations of ATP and H2O2 were estimated immediately by the reduction of the amperometric signal in response to decomposition of detected molecules by the addition of apyrase-converting (ATP) or catalase-converting (H2O2) enzymes. Application of apyrase revealed the near absence of ATP signal in the urine, suggesting rapid conversion of endogenous ATP to other purinergic products in the kidney (n=5 rats). The urinary ATP concentrations in 2 rats were 135 and 181 nmol/L, and the signal was significantly lower than the detection limit in 3 other rats.

Figure 6A illustrates the catalase-dependent H2O2 amperometric signal of the biosensor in response to a 30-mm Hg increase in RPP. Values obtained for H2O2 concentration at low pressures were similar to those recorded previously by either microdialysis or biosensor amperometry in isolated perfused kidneys. A comparable increase in urinary H2O2 in response to changes in RPP was also reported previously using a fluorescence spectrometric assay.

Discussion

Purinergic signaling is recognized as having an important role in the regulation of renal and cardiovascular function. In this study, a novel approach was applied for in vivo measurement of ATP concentrations in the kidney. We used microelectrode biosensors that incorporate enzymatic sensing elements to selectively detect, by amperometry techniques, small amounts of physiologically relevant molecules.

The studies represent the first application of such biosensors to measure in vivo renal-tissue ATP levels in blood-perfused kidneys. Studies using microdialysis approaches have previously revealed a direct relationship among changes in RPP, tissue ATP levels, and sodium excretion. Specifically, Nishiyama, Majid, and Navar, utilizing acutely implanted dialysis tubes, were the first to observe elevations of tissue ATP in response to increases of RPP and hypothesized a link between purine release and pressure natriuresis. in the initial study by Nishiyama et al, a direct relationship between autoregulatory adjustments of renal vascular resistance and renal interstitial ATP concentrations was observed. Other studies revealed that ATP contributed to autoregulatory adjustments of renal vascular resistance that were mediated by changes in the feedback response of the tubuloglomerular feedback mechanism.

The application of biosensors in the present study has advanced our understanding of these relationships by providing the important in vivo kinetic associations among step changes in RPP, ATP production, and sodium excretion that could not be obtained by microdialysis or in studies that assessed tubuloglomerular feedback. The results showed that cortical ATP concentrations at the tip of the sensor rise significantly, within 5 minutes, in response to changes in RPP. Such changes, in the µmol/L range, are known to be sufficient to acutely stimulate most of the purinergic receptors and are consistent with a physiological role of purinergic signaling in the regulation of pressure natriuresis. Analysis of urine showed nearly undetectable levels of ATP in response to changes in RPP, consistent with the knowledge that tissue ATP is rapidly converted to adenosine and, further, to uric acid.

Multiple studies in isolated nephron segments have demonstrated the relationship between tubular flow and epithelial cell ATP release, both constitutively and in response.
to physiological stimuli\textsuperscript{8,32,33} such as mechanical and shear stress.\textsuperscript{18,34} ATP is recognized as affecting tubular sodium transport mechanisms via the stimulation of ionotropic (P2X) and metabotropic G protein-coupled (P2Y) purinergic receptors.\textsuperscript{9,35,36} The P2Y\textsubscript{2} receptor is one of the most thoroughly characterized P2 receptors in the cortical collecting ducts (CCDs),\textsuperscript{7,8,37} in which the fine tuning of sodium reabsorption occurs. In isolated mouse and rabbit CCDs, the expression of P2Y\textsubscript{2} has been described for both the apical and basolateral membranes.\textsuperscript{33,38} Stimulation of P2Y\textsubscript{2} receptors by ATP causes the activation of phospholipase C and the hydrolysis of phosphatidylinositol 4,5-bisphosphate. Activation of this pathway has been shown to inhibit the epithelial sodium channel (ENaC), thereby reducing sodium reabsorption promoting natriuresis.\textsuperscript{39,40} Notably, in split-open murine collecting ducts, scavenging of endogenous ATP and the inhibition of P2 receptors in the absence of exogenous stimuli resulted in a rapid increase in ENaC activity.\textsuperscript{16} These results, in combination with studies showing that tubular ATP concentrations are in the range of 0.2 to 0.3 \textmu mol/L, suggest that ATP tonically inhibits ENaC. Moreover, during the consumption of a high salt diet, P2Y\textsubscript{2} stimulation has been shown to increase urinary sodium excretion by inhibiting the activity of ENaC.\textsuperscript{41}

The loss of ATP inhibition of ENaC would be predicted to alter the pressure-natriuretic relationship, to lead to sodium retention, and to promote hypertension. Indeed, global knockout of P2Y\textsubscript{2} receptors in mice resulted in volume expansion and hypertension, particularly when aldosterone levels were augmented.\textsuperscript{42,43} In addition to the critical role of P2Y\textsubscript{2} receptors in the control of water and electrolyte balance in the kidney, contributions of other P2 receptors have also been proposed. The role of the P2Y\textsubscript{12} receptor in the CCDs, for instance, especially during lithium-induced nephrogenic diabetes insipidus, has been reported.\textsuperscript{15,44} Other ion channels and transporters also might be under control of ATP released in response to changes in RPP, either directly or indirectly. It is recognized, for instance, that luminal flow and extracellular ATP can stimulate the production of nitric oxide in the thick ascending limbs,\textsuperscript{45} which in turn modifies NKCC2 (Na\textsuperscript{+}/K\textsuperscript{+}/2Cl\textsuperscript{−} cotransporter) activity.\textsuperscript{42,46}

Recent studies indicate an important pathophysiological role of purinergic signaling in multiple kidney diseases

Figure 5. Summary of pressure natriuresis/diuresis response in Sprague–Dawley rats following an increase in renal perfusion pressure (RPP). Changes in RPP induced by ligations around the celiac/superior mesenteric arteries (steps 1 and 2). Controls 1, 2, and 3 represent either basal condition (control 1) or phase after ligation removal. MAP was monitored via the carotid artery (A). Urine was collected bilaterally from both ureter catheters. Shown are the summary of changes in urine flow (B), Na\textsuperscript{+} excretion (C), and K\textsuperscript{+} excretion (D). The differences in the mean values among the groups were tested by a repeated measures ANOVA (n≥7; from N=8 rats; P≤0.001). All pairwise multiple comparison procedures (Holm–Sidak method) with overall significance level are indicated; *P<0.03 or ***P<0.001.
including hypertension, polycystic kidney, and diabetes mellitus.9,47–49 In most of these conditions, ATP production and secretion has been found to be elevated. Modifications of purinergic receptor composition have also been reported that redirect metabotropic to ionotropic pathways in diseases such as hypertension and polycystic kidney diseases, in which expression of P2X4 and P2X7 ionotropic receptors are altered.47,50,51 Both receptors require higher levels of ATP for activation, which is the key component for pathological transformation. The application of biosensors to such studies would enable one to explore not only the physiological role of dynamic concentrations of cortical ATP and its derivatives but also the mechanisms and primary causes of renal purinergic imbalances in pathophysiological conditions.

The molecular mechanisms related to ATP release remain unclear; however, evidence shows that in the CCDs, mechanically stimulated connexin 30 hemichannels (Cx30) are a primary source of ATP release into the lumen.52,53 In isolated microperfused CCDs, increased tubular flow was found to stimulate ATP release from intercalated cells, a response nearly abolished in tubules isolated from Cx30 knockout mice. Highlighting the central role of the channel in flow-stimulated ATP release, it was found that deletion of Cx30 in mice impaired natriuretic responses to step increases in arterial pressure and resulted in ENaC-dependent, salt-sensitive hypertension.53 These data corroborate those presented in the current study, implicating ATP as a key regulator of the pressure-natriuresis mechanism.

In addition to changes in RPP, other mechanisms may result in ATP release. One such mechanism is angiotensin II stimulation of ATP. Previous studies in our laboratory revealed that angiotensin II could induce a rapid release of ATP in the isolated kidney, a response that was strongly enhanced in Dahl salt-sensitive rats fed a high-salt diet.25 Another mechanism that may stimulate ATP release and sodium excretion in response to an increase of RPP could be the proposed crosstalk between purinergic and endothelin signaling.54 Consistent with this, ATP release and P2 receptor stimulation were shown to be upstream events of the flow-induced increases in endothelin expression and activity in the CCDs55 both of which play significant roles in the regulation of ENaC activity.56,57

In summary, the goal of this study was to determine the role of ATP in the pressure-natriuresis mechanism. The relationship between arterial blood pressure and renal sodium excretion is well established. Guyton and colleagues were the first to hypothesize that increased sodium and water excretion, in the face of elevated blood pressure, could serve as an important mechanism by which blood volume decreased in an attempt to restore blood pressure to control levels.1 In the present study, we showed that step increases of RPP result in acute ATP release in concentrations known to reduce ENaC activity in the CCDs and natriuresis. This negative feedback control mechanism would be expected to produce a rightward shift in the renal function curve observed in both clinical and experimental hypertension. Studies by others indicate that ATP-driven mechanisms such as tubuloglomerular feedback would also contribute to this shift of the pressure-natriuresis relationship as a consequence of ATP release across the basolateral membrane of macula densa cells.13,58,59 It is
recognized that ATP release from these cells is regulated by luminal NaCl concentration and dietary salt intake. The close correlation of changes in RPP and cortical ATP concentrations with sodium excretion, together with known effects of ATP on regulation of glomerular filtration and tubular transport, supports an important role of ATP release in the pressure-natriuresis mechanism.

Perspectives

We presented a novel method that enables real-time in vivo measurements of ATP, adenosine, H2O2, and other endogenous substances not only in the kidney but other organs. Such kinetic data of ATP provide critical details of cellular signaling and can reveal novel insights into signaling pathways that play important roles in kidney function and are related to pharmacological agents. Although several types of biosensors are sensitive to various endogenous substances, few are suitable for use in blood-perfused organs. The gold biosensors applied in this study are suitable for blood-perfused kidneys, although they are limited to measurement of ATP, adenosine, inosine, and hypoxanthine. We assessed ATP release in the kidney, but it will be important in the future to test other sensors. The simultaneous application of 2 types of biosensors would be of interest for future studies and would provide novel information about the dynamics of purinergic signaling in the kidney. Extracellular ATP, for instance, is rapidly catabolized by ectonucleotidases and subsequently metabolized to adenosine. Application of both ATP and adenosine sensors would allow this transient ATP release and its conversion to adenosine to be quantified in real time in vivo. However, even the application of a single sensor, as used in the present study, can reveal the kinetics of the relationship between ATP production and a given physiological stimulus and response.

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Disclosures

None.

References

1. Guyton AC, Coleman TG, Granger HJ. Circulation: overall regulation. Annu Rev Physiol. 1972;34:13-46.
2. Cowley AW Jr. Long-term control of arterial blood pressure. Physiol Rev. 1992;72:231–300.
3. Guyton AC. Blood pressure control-special role of the kidneys and body fluids. Science. 1991;252:1813–1816.
4. Hall JE. Renal dysfunction, rather than nonrenal vascular dysfunction, mediates salt-induced hypertension. Circulation. 2016;133(8):894–906.
5. Majid DS, Insoho EW, Navar LG. P2 purinoceptor saturation by adenosine triphosphate repairs renal autoregulation in dogs. J Am Soc Nephrol. 1999;10:492–498.
6. Burnstock G. Purinergic signaling in the cardiovascular system. Circ Res. 2017;120:207–228.
7. Burnstock G, Evans LC, Bailey MA. Purinergic signalling in the kidney in health and disease. Purinergic Signal. 2014;10:71–101.
8. Mironova E, Boiko N, Bugaj V, Kucher V, Stockand JD. Regulation of Na+ excretion and arterial blood pressure by purinergic signalling intrinsic to the distal nephron: consequences and mechanisms. Acta Physiol. 2015;213:213–221.
9. Solini A, Usuelli V, Fiorina P. The dark side of extracellular ATP in kidney diseases. J Am Soc Nephrol. 2015;26:1007–1016.
10. Guan Z, Insoho EW. Role of adenosine 5’-triphosphate in regulating renal microvascular function and in hypertension. Hypertension. 2011;58:333–340.
11. Insoho EW, Cook AK, Imig JD, Vial C, Evans RJ. Physiological role for P2X1 receptors in renal microvascular autoregulatory behavior. J Clin Invest. 2003;112:1895–1905.
12. Insoho EW, Cook AK, Navar LG. Pressure-mediated vasoconstriction of juxtamedullary afferent arterioles involves P2-purinoceptor activation. Am J Physiol. 1996;271:F1077–F1085.
13. Bell PD, Komlosi P, Zhang ZR. ATP as a mediator of macula densa cell signalling. Purinergic Signal. 2009;5:461–471.
14. Nishiyama A, Navar LG. ATP mediates tubuloglomerular feedback. Annu Rev Physiol. 2007;69:297–321.
15. Kishore BK, Carlson NG, Ecelbarger CM, Kohan DE, Muller CE, Nelson RD, Peti-Peterdi J, Zhang Y. Targeting renal purinergic signalling for the treatment of lithium-induced nephrogenic diabetes insipidus. Acta Physiol. 2015;214:176–188.
16. Pochnuyk O, Bugaj V, Rieg T, Insel PA, Mironova E, Vallon V, Stockand JD. Paracrine regulation of the epithelial Na+ channel in the mammalian collecting duct by purinergic P2Y2 receptor tone. J Physiol. 2003;546:195–207.
17. Prætorius HA, Leiziger J. Released nucleotides amplify the cilium-dependent, flow-induced [Ca2+]i response in MDCK cells. Acta Physiol. 2009;197:241–251.
18. Jensen ME, Øgaard E, Christensen MH, Prætorius HA, Leiziger J. Flow-induced [ca2+]i increase depends on nucleotide release and subsequent purinergic signaling in the intact nephron. J Am Soc Nephrol. 2007;18:2062–2070.
19. Bell PD, Lapointe JY, Sabirov R, Hayashi S, Peti-Peterdi J, Manabe K, Kovacs G, Okada Y. Macula densa cell signalling involves ATP release through a maxi anion channel. Proc Natl Acad Sci USA. 2003;100:4322–4327.
20. Lu D, Insel PA. Hydrolysis of extracellular ATP by ectonucleoside triphosphate diphosphohydrolase (ENTPD) establishes the set point for fibrotic activity of cardiac fibroblasts. J Biol Chem. 2013;288:19040–19049.
21. Anulkumar N, Turner CM, Sixma ML, Singer M, Unwin R, Tam FW. Purinergic signaling in inflammatory renal disease. Front Physiol. 2013;4:194.
22. Nishiyama A, Jackson KE, Majid DS, Rahaman M, Navar LG. Renal interstitial fluid ATP responses to arterial pressure and tubuloglomerular feedback activation during calcium channel blockade. Am J Physiol Heart Circ Physiol. 2006;290:H772–H777.
23. Nishiyama A, Majid DS, Taher KA, Miyatake A, Navar LG. Relation between renal interstitial ATP concentrations and autoregulation-mediated changes in renal vascular resistance. Circ Res. 2000;86:656–662.
24. Nishiyama A, Majid DS, Walker M III, Miyatake A, Navar LG. Renal interstitial ATP responses to changes in arterial pressure during alterations in tubuloglomerular feedback activity. Hypertension. 2001;37:753–759.
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25.  Palygin O, Levchenko V, Ilatovskaya DV, Pavlov TS, Ryan RP, Cowley AW Jr, Staruschenko A. Real-time electrochemical detection of ATP and H₂O₂ release in freshly isolated kidneys. Am J Physiol Renal Physiol. 2013;305:F134–F141.

26.  Palygin O, Levchenko V, Evans LC, Blass G, Cowley AW Jr, Staruschenko A. Use of enzymatic biosensors to quantify endogenous ATP or H₂O₂ in the kidney. J Vis Exp. 2015;104:e53059.

27.  Tian F, Llaudet E, Dale N. Ruthenium purple-mediated microelectrode biosensors based on sol-gel film. Anal Chem. 2007;79:6760–6766.

28.  Lalo U, Palygin O, Rasooli-Nejad S, Andrew J, Haydon PG, Pankratov Y. Excytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex. PLoS Biol. 2014;12:e1001747.

29.  Jin C, Hu C, Polichnowski A, Mori T, Skelton M, Ito S, Cowley AW Jr. Effects of renal perfusion pressure on renal medullary hydrogen peroxide and nitric oxide production. Hypertension. 2009;53:1048–1053.

30.  Gourine AV, Llaudet E, Dale N, Spyker KM. Release of ATP in the ventral medulla during hypoxia in rats: role in hypoxic ventilatory response. J Neurosci. 2005;25:1211–1218.

31.  Dale N, Frenquelli BM. Measurement of purge release with microelectrode biosensors. Purinergic Signal. 2012;8:27–40.

32.  Praetorius HA, Leipziger J. Intrarenal purinergic signaling in the control of renal tubular transport. Annu Rev Physiol. 2010;72:377–393.

33.  Vallon V, Rieg T. Regulation of renal nacl and water transport by the ATP/UTP/P2Y2 receptor system. Am J Physiol Renal Physiol. 2011;301:F463–F475.

34.  Holtzclaw JD, Cornelius RJ, Hatcher LI, Sansom SC. Coupled ATP and potassium efflux from intercalated cells. Am J Physiol Renal Physiol. 2011;300:F1319–F1326.

35.  Menzies RI, Staruschenko A. Macula densa volume and luminal solute concentration/osmolality. Kidney Int. 2017;91:315–323.

36.  Van Beusem J, Insel PA. Regulation of renal function and blood pressure control by P2 purinoreceptors in the kidney. Curr Opin Pharmacol. 2015;21:82–88.

37.  Deetjen P, Thomas J, Lehrmann H, Kim SJ, Leipziger J. The luminal P2Y2 receptor in the isolated perfused mouse cortical collecting duct. J Am Soc Nephrol. 2000;11:1798–1806.

38.  Bailey MA, Shirley DG. Effects of extracellular nucleotides on renal tubular solute transport. Purinergic Signal. 2009;5:473–480.

39.  Kunzelmann K, Bachhuber T, Regeer R, Markovich D, Sun J, Schreiber R. Purinergic inhibition of the epithelial Na⁺ transport via hydrolysis of PIP2. FASEB J. 2005;19:142–143.

40.  Ma HP, Li L, Zhou Z, Eaton DC, Warnock DG. ATP masks stretch activation of epithelial Na⁺ transport by enhancing apical P2Y2-receptor coupling. Am J Physiol Renal Physiol. 2004;286:F1054–F1060.

41.  Pochynyuk O, Rieg T, Bugaj V, Stockand JD, Mironova E, Vandewalle A, Pochynyuk O, Sorokin A, Staruschenko A. Macula densa cell volume and luminal solute concentration/osmolality. Kidney Int. 2006;70:865–871.

42.  Aluksanasuwan S, Suetsakit K, Fong-Ngern K, Thongboonkerd V. Role of HSPI60 (HSPD1) in diabetes-induced renal tubular dysfunction: regulation of intracellular protein aggregation, ATP production, and oxidative stress. FASEB J. 2017;31:2157–2167.

43.  Zhang Y, Listhrop R, Ecelbarger CM, Kishore BK. Renal sodium transporter/stimulated ATPase activity in macula densa cells of the rabbit kidney. Am J Physiol Renal Physiol. 2010;305:F541–F552.

44.  Palygin O, Rieg T, Bugaj V, Stockand JD, Mironova E, Vandewalle A, Pochynyuk O. Macula densa cell volume and luminal solute concentration/osmolality. Kidney Int. 2006;70:865–871.

45.  Casabrienda C, Niskanen KO, D’Souza P. ATP mediates flow-induced NO production in thick ascending limbs. Am J Physiol Renal Physiol. 2012;303:F194–F200.

46.  Zhang Y, Listhrop R, Ecelbarger CM, Kishore BK. Renal sodium transporter/stimulated ATPase activity in macula densa cells of the rabbit kidney. Am J Physiol Renal Physiol. 2010;305:F541–F552.

47.  Ilatovskaya DV, Palygin O, Staruschenko A. Functional and therapeutic importance of purinergic signaling in polycystic kidney disease. Am J Physiol Renal Physiol. 2016;311:F1135–F1139.

48.  Franco M, Bautista-Perez R, Perez-Mendez O. Purinergic receptors in tubulointerstitial inflammatory cells: a pathophysiological mechanism of salt-sensitive hypertension. Acta Physiol. 2015;214:75–87.

49.  Palygin O, Staruschenko A. Macula densa cell volume and luminal solute concentration/osmolality. Kidney Int. 2006;70:865–871.

50.  Menzies RI, Unwin RJ, Bailey MA. Renal P2 receptors and hypertension. Acta Physiol. 2015;213:232–241.

51.  Menzies RI, Unwin RJ, Dash RK, Beard AW, Cowley AW, Carlson BE, Mullins JJ, Bailey MA. Effect of P2X4 and P2X7 receptor antagonism on the pressure diuresis relationship in rats. Front Physiol. 2013;4:305.

52.  Mironova E, Peti-Peterdi J, Bugaj V, Stockand JD. Diminished paracrine regulation of the epithelial Na⁺ channel by purinergic signaling in mice lacking connexin 30. J Biol Chem. 2011;286:1054–1060.

53.  Sipos A, Vargas SL, Toma I, Hanner F, Willecke K, Peti-Peterdi J. Connexin 30 deficiency impairs renal tubular ATP release and pressure natriuresis. J Am Soc Nephrol. 2009;20:1724–1732.

54.  Gohar EY, Kasztan M, Pollock DM. Interplay between renal endothelin and purinergic signaling systems. Am J Physiol Renal Physiol. 2017. [published online ahead of print Feb, 2017] DOI: 10.1152/ajpregg.00639.2016.

55.  Pandit MM, Insoo EW, Zhang S, Seki T, Rohatgi R, Gusella L, Kishore B, Kohan DE. Flow regulation of endothelin-1 production in the inner medullary collecting duct. Am J Physiol Renal Physiol. 2004;286:F1054–F1060.

56.  Pavlov TS, Chadri A, Ilatovskaya DV, Levchenko V, Vandewalle A, Pochynyuk O, Sorokin A, Staruschenko A. Macula densa cell volume and luminal solute concentration/osmolality. Kidney Int. 2006;70:865–871.

57.  Bugaj V, Pochynyuk O, Mironova E, Vandewalle A, Medina JL, Stockand JD. Regulation of the epithelial Na⁺ channel by endothelin-1 in rat collecting duct. Am J Physiol Renal Physiol. 2008;295:F1063–F1070.

58.  Schnermann J. Maintained tubuloglomerular feedback responses during acute inhibition of P2 purinergic receptors in mice. Am J Physiol Renal Physiol. 2011;300:F339–F344.

59.  Schnermann J, Marver D. ATPase activity in macula densa cells of the rabbit kidney. Pflügers Arch. 1986;407:82–88.

60.  Komlosi P, Peti-Peterdi J, Fuson AL, Firtha A, Rosiwall L, Bell PD. Macula densa basolateral ATP release is regulated by luminal [NaCl] and dietary salt intake. Am J Physiol Renal Physiol. 2004;286:F1054–F1058.