Juxtamedullary Afferent Arteriolar Responses to P₁ and P₂ Purinergic Stimulation

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We assessed the responsiveness of rat juxtamedullary afferent arterioles to purinergic stimulation using the in vitro blood-perfused juxtamedullary nephron technique combined with videomicroscopy to allow direct measurement of arteriolar inside diameter. To minimize the contribution of endogenously formed angiotensin II, all rats were pretreated with enalaprilat (2 mg i.v.) for 30 minutes before the right kidney was isolated and prepared for study. Renal perfusion pressure was set at 110 mm Hg and held constant. Afferent arteriolar diameter averaged 20.9±0.8 μm (n=41) under control conditions. Exposure to 1.0 μM 2-chloroadenosine induced a significant (11.1±3.2%) reduction in vessel diameter, whereas a 100 μM concentration induced an afferent vasodilation (7.6±1.5%; p<0.05). These data are consistent with the preferential stimulation of high affinity constrictor adenosine receptors (A₁) at lower concentrations and activation of lower affinity vasodilator adenosine receptors (A₂) at higher concentrations. In contrast, ATP elicited a significant afferent vasoconstriction of approximately 9.2%, 12.9%, and 10.0% at concentrations in the range of 1-100 μM (p<0.05). Treatment with ADP, at concentrations up to 100 μM, failed to alter vessel caliber significantly. Furthermore, the nonhydrolyzable ATP analogue α,β-methylene ATP produced a rapid and potent vasoconstriction, which mimicked the response to ATP. These data reveal the presence of both adenosine-sensitive P₁ and ATP-sensitive P₂ purinergic receptors on rat juxtamedullary afferent arterioles and demonstrate that ATP can induce afferent arteriolar vasoconstriction directly without first requiring hydrolysis to adenosine. (Hypertension 1991;17:1033–1037)

In recent years, the purinergic agonist adenosine has received considerable attention as a potential regulator of renal microvascular hemodynamics. An early report by Oswald et al. postulated a role for adenosine in the transmission of tubuloglomerular feedback signals altering renal vascular resistance. However, the fact that adenosine can act as either a vasoconstrictor or a vasodilator of the renal microvasculature has led to some confusion over exactly how adenosine would fit into a scheme of physiological regulation. It also is unclear whether adenosine might be the only purinergic agonist capable of altering renal microvascular tone. Another major purinergic agonist, ATP, has been shown to exert potent stimulatory effects in other tissues, including vascular smooth muscle and endothelial cells when present in the extracellular space. ATP has been shown to stimulate prostaglandin production and polyphosphoinositide hydrolysis in rat mesangial cells...
left carotid artery. Blood from the donor rat was prepared according to a previously published procedure.16-18 Blood was centrifuged at 4°C, and the white blood cell fraction was discarded. The plasma concentration of ionized calcium was adjusted to 1.0 mM with calcium chloride. Plasma colloid osmotic pressure was adjusted to 18 mm Hg with the addition of bovine serum albumin (BSA), and the plasma was passed through 5.0- and 0.22-μm filters and mixed with erythrocytes to yield a hematocrit of 33%. The reconstituted blood was filtered through a 5-μm nylon mesh and oxygenated in a closed reservoir pressurized with a 95% O₂-5% CO₂ gas mixture.

The right renal artery of the kidney donor rat was cannulated via the superior mesenteric artery and immediately perfused with a Tyrode’s buffer containing 52 g/l BSA (Sigma Chemical Co., St. Louis) at pH 7.4.17 The perfused kidney was removed and sectioned longitudinally, leaving the papilla intact on the dorsal two-thirds portion of the organ. Small incisions were made into the lateral fornices, and the papilla was reflected back to expose the underlying pelvic cavity. The pelvic mucosa, adipose, and connective tissues overlying the inner cortical surface were removed, thus exposing the main branches of the renal artery, tubules, glomeruli, and related microvasculature of juxamedullary nephrons. Tight ligatures were placed around the distal ends of the large arterial vessels supplying superficial interlobular arteries and afferent arterioles. On completion of all microdissection procedures, the perfusate was replaced with the reconstituted blood. The blood perfusate was stirred continuously in a closed reservoir and pressurized by a tank containing a 95% O₂-5% CO₂ gas mixture. A pressure cannula, fixed at the tip of a double-barreled perfusion cannula, was connected to a Statham P23Db pressure transducer linked to a calibrated polygraph channel (Grass Instruments, Quincy, Mass.) to provide a continuous record of perfusion pressure. Renal perfusion pressure was set and maintained at 110 mm Hg by adjusting the regulator controlling the flow of gas from the tank to the blood reservoir. Sensitive control of perfusion pressure was possible by adjusting the regulator settings. The inner cortical surface of the kidney was superfused continuously with warmed (37°C) Tyrode’s buffer containing 10 g/l BSA.

Afferent arteriolar inside diameter was measured using previously described videometric techniques.16-18 The perfusion chamber containing the prepared kidney was affixed to a movable stage of a Leitz Laborlux 12FS microscope (Wild Leitz USA, Inc., Rockleigh, N.J.) equipped with long working distance achromatic dry objectives (×10, ×20, ×32) and a water immersion objective (×40). The tissue was transilluminated, and the focused image was transferred via a high-resolution Newvicon camera (NC67m, Dage-MTI, Inc., Michigan City, Ind.) through an image-enhancing processor (MFJ-1425, MFJ Enterprises Inc., Starkville, Miss.) and was displayed on a videomonitor. The video signal was recorded simultaneously on videotape for later analysis. Vascular inside diameter was measured at a single site using an image-shearing monitor (model 901, Instrumentation for Physiology & Medicine, Inc., San Diego, Calif.) calibrated with a stage micrometer. This system results in diameter measurements that are reproducible to within 1 μm. Afferent arterioles were selected for study on the basis of clear visibility of both vessel walls and an established connection to a glomerulus. Only vessels with rapid flow of erythrocytes were studied because these vessels were assumed to be representative of the normal nephron population. If the passage of single erythrocytes could be followed along the vessel lumen, the vessel was rejected.

**Experimental Protocol**

After a 15-minute equilibration period, an experimental protocol was initiated consisting of consecutive 5-minute treatment periods. Treatments were administered by bathing the tissue with a superfusate solution with varying composition. After an initial control period, the tissue was exposed to increasing concentrations of 2CA, ADP, or ATP over the range of 0.1, 1.0, 10, and 100 μM, followed by a final recovery period. In a separate experimental series, the afferent arteriolar response to the nonhydrolyzable ATP analogue α,β-methylene ATP was determined. The experiments were conducted as described above except that the tissue was exposed to only a single concentration (1.0 μM) of the agonist. Afferent arteriolar diameter was measured at 12-second intervals throughout the entire protocol. Plateau diameter responses to experimental manipulations were obtained during the final 2 minutes of the 5-minute treatment period.

**Drugs**

Enalaprilat was a gift from Merck Sharp & Dohme, Rahway, N.J. ATP, ADP, and α,β-methylene ATP were obtained from Sigma Chemical Co. 2CA was obtained from Research Biochemicals, Inc., Natick, Mass.

**Statistical Analysis**

Data from each experimental protocol were evaluated using an analysis of variance for repeated measures. Differences between group means within each protocol were determined using Newman-Keuls multiple range test (PC ANOVA software package; Human Systems Dynamics, Northridge, Calif.). Values of p<0.05 were considered statistically significant. All values are reported as mean±SEM.

**Results**

The afferent arteriolar response to stimulation with 2CA, a nonspecific, nonhydrolyzable adenosine receptor agonist, is shown in Figure 1. Afferent arteriolar diameter averaged 21.3±1.6 μm during the control period. Topical administration of 2CA reduced afferent diameter to 20.6±1.7 and 19.0±1.7 μm (p<0.05) at concentrations of 0.1 and 1.0 μM,
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Figure 1. Line graph showing afferent arteriolar response to 2-chloroadenosine. n, Number of vessels. *p<0.05 vs. control.

respectively; however, as the concentration of 2CA increased beyond 1.0 μM, the afferent vasoconstriction reverted to a vasodilation. Vessel diameter increased to 20.0±1.7 and then 23.0±1.9 μm (p<0.05) at concentrations of 10 and 100 μM, respectively. Cessation of 2CA treatment restored vessel diameter to 21.0±1.9 μm or 98.2±2.4% of control (p>0.05).

The response of juxtamedullary afferent arterioles to ATP is shown in Figure 2. Afferent arteriolar diameter averaged 20.2±2.7 μm during the control period. Subsequent exposure to increasing concentrations of ATP elicited a significant vasoconstriction with 1.0 μM ATP, which was sustained at concentrations of 10 and 100 μM, respectively. Removal of ATP from the bathing medium resulted in a full recovery, with vessel caliber returning to 19.8±2.6 μm (98.4±0.7% of control; p>0.05).

The vasoconstrictor response to ATP could be mediated either by ATP acting directly at its receptor or through hydrolysis of ATP through the intermediates ADP and AMP to adenosine. To evaluate this possibility, the afferent arteriolar response to ADP was determined (Figure 2). Afferent diameter averaged 19.8±1.4 μm during the control period and remained unchanged in the presence of ADP. Vessel inside diameter averaged 20.3±1.7 μm at both 0.1 and 1.0 μM ADP and 19.1±1.5 μm with 10 and 100 μM ADP, respectively. These values did not differ significantly from control (p>0.05).

The time course of the afferent arteriolar response to ATP and 2CA is presented in Figure 3. During the control period, afferent diameter remained stable at 22.3±1.9 and 21.9±1.5 μm for the 2CA (n=6) and ATP (n=5) groups, respectively. Exposure to 1.0 μM 2CA induced a slow, steady vasoconstriction that reached a minimum vessel caliber after approximately 2 minutes and remained at that diameter through the remaining 3 minutes of the treatment period. Afferent diameter averaged 17.7±1.6 μm in the presence of 2CA (p<0.05 versus control). In contrast, the response to 10 μM ATP produced a rapid reduction in vessel caliber, reaching a minimum diameter of 15.9±0.9 μm within 0.6 minutes. This sharp reduction in vessel diameter waned slowly over the next 2 minutes to achieve a steady-state diameter of 19.2±1.5 μm, which was still significantly smaller than control (p<0.05). In both groups, afferent diameter returned to within 99% of control during the recovery period (p>0.05).

The effects of α,β-methylene ATP were evaluated to confirm the ability of ATP to stimulate afferent vasoconstriction directly. Methylene (—CH₂—)-substituted ATP analogues are inhibitors of ecto 5'-nucleotidase, and therefore, their vascular effects are P₂ purinergic receptor–specific rather than occurring through hydrolysis to adenosine.¹¹ The time course of the response to α,β-methylene ATP is contrasted with that of 2CA and is illustrated in Figure 3. α,β-Methylene ATP, at a concentration identical to that of 2CA (1 μM), caused a sharp decline in

Figure 2. Line graph showing afferent arteriolar response to ATP and ADP. n, Number of vessels. *p<0.05 vs. control.

Figure 3. Line graph showing time course of afferent arteriolar response to 2-chloroadenosine (2CA), ATP, and α,β-methylene ATP applied at the 5-minute time point. Each point represents mean vessel diameter measured at 12-second intervals. Standard error bars have been omitted for clarity. Numbers in parentheses indicate number of arterioles in each group.
afferent arteriolar diameter from a stable 22.1±2.5 to 8.0±1.3 μm within 0.6 minutes of exposure (n=7). This 64% reduction in vessel diameter waned rapidly and achieved a steady-state diameter of 20.5±2.3 μm, or 7.1±4.8% below control (p<0.05). As previously noted, continued exposure to α,β-methylene ATP can lead to desensitization of the P2 purinergic receptor and results in a waning of the response by smooth muscle.8 Removal of α,β-methylene ATP from the superfusate resulted in a complete recovery of afferent diameter to 22.6±2.6 μm, or 102.4±4.1% of control (p>0.05).

Discussion

The present study was designed to characterize the afferent arteriolar responses to P2 purinergic stimulation with ATP and to contrast that response with one produced by the adenosine analogue 2CA. Experiments were conducted using the in vitro blood-perfused juxtamedullary nephron technique, because this method permits direct visualization of rat renal microvascular elements while maintaining intact anatomical and functional glomerular–tubular relations.15 Previous studies have verified that glomerular filtration and proximal tubular reabsorption proceed at rates comparable with those measured in vivo.15 Similarly, renal microvascular responsiveness to angiotensin II, norepinephrine, and other circulating or locally formed vasoactive substances has been demonstrated.16-18

The P2 class of purinergic receptors is defined by a relative preference to bind adenosine over other adenine nucleotides such as ATP, ADP, or AMP.1,2,9 This general receptor class is further divided into two receptor subtypes, A1 and A2. In most tissues, stimulation of A1 receptors leads to inhibition of adenylate cyclase, whereas A2 receptors are linked to activation of the enzyme.1,2,20 In vascular smooth muscle, stimulation of A2 receptors leads to vasoconstriction, whereas adenosine binding to A2 receptors leads to vasorelaxation. Analysis of the present studies with 2CA indicates that both adenosine A1 and A2 receptor subtypes are present on rat juxtamedullary afferent arterioles. This conclusion is based on the observation that exposure of afferent arterioles to increasing concentrations of 2CA leads to a biphasic response. Initially, 2CA induces an afferent vasoconstriction, presumably through coupling with A1 (vasoconstrictor) receptors that exhibit a high affinity for adenosine.1,2,9 However, as the concentration of 2CA increases, the vasoconstrictor response is supplanted by a vasodilation indicative of stimulation of vasodilator adenosine A2 receptors.

P2 purinergic receptors exhibit a higher selectivity for ATP than for adenosine.1,9,10 In the present study, 1 μM ATP produced a significant afferent arteriolar vasoconstriction. This concentration agrees well with the typical threshold for ATP-induced activity determined in numerous other tissues.1,10 Further increases in ATP concentration to 10 or 100 μM also produced sustained vasoconstrictor responses, in contrast to the vasodilation seen with 100 μM 2CA. Because of the difference between the concentration response profiles of ATP and 2CA, it would seem that two different mechanisms are involved. Furthermore, the observation that ADP failed to elicit a response, whereas the nonhydrolyzable ATP analogue α,β-methylene ATP produced responses mimicking those of ATP, supports the contention that ATP induces afferent vasoconstriction by direct interaction with P2 purinergic receptors, rather than first requiring hydrolysis to adenosine. The results of the present studies establish the presence of both vasoconstrictor and vasodilator P1 receptors as well as vasoconstrictor P2 receptors on afferent arterioles of rat juxtamedullary nephrons.

Determining the physiological significance of renal purinergic receptors will require additional study. The renal adenosine system already has received considerable attention, and yet its significance still remains clouded. Recently, it was reported that adenosine A1 receptor agonists added to luminal perfusates decreased stop-flow pressure4 or could augment the tubuloglomerular feedback–mediated reduction in stop-flow pressure induced by increasing loop perfusion rate.3 The tubuloglomerular feedback responses were increased during loop perfusion with low concentrations of A1 agonists but appeared to be attenuated when higher concentrations were used.3 Addition of adenosine A2 receptor agonists or adenosine at high concentrations also attenuated stop-flow pressure responses. Furthermore, administration of intraluminal adenosine receptor antagonists either blunted or blocked tubuloglomerular feedback–induced reductions in stop-flow pressure.4,5 The disparity in the A1 responses at low and high doses could reflect the lack of exclusivity of the agonist for A1 receptors, thus permitting interaction with A2 receptors at higher concentrations. Such an explanation is consistent with the biphasic response of juxtamedullary afferent arterioles to the nonspecific adenosine agonist 2CA seen in the present studies.

The effects of ATP have been evaluated in a number of different tissues, including vascular smooth muscle from several vascular beds.1,9-14,19 However, there are few data describing renal responses to ATP. A recent report indicated that rat mesangial cells respond to ATP by enhancing polyphosphoinositide hydrolysis as well as prostaglandin E2 synthesis.13 Inositol phosphate formation is known to be involved in elevating intracellular calcium concentration, which, in vascular smooth muscle, would translate into a vasconstrictor response.21 Similarly, exposure of renal microvascular tissue to prostaglandin E2 in the extravascular fluid space can induce afferent arteriolar vasoconstriction.17 Whether the responses to ATP observed in the present study occur through direct effects on renal arteriolar smooth muscle or through stimulation of a secondary system remains to be determined. However, the rapidity with which ATP and α,β-methylene ATP
exert their effects suggests that these agents are acting directly on renal microvascular smooth muscle. One possible mechanism for such direct interaction involves stimulation of receptor-operated calcium channels in vascular smooth muscle.12,14 Further experimentation is needed to determine whether ATP exerts its effects through opening calcium channels, through inositol phosphate-mediated calcium mobilization, or through stimulation of some other secondary signal. In any case, the present data raise the possibility that P2 purinergic receptors may represent a previously unappreciated means by which renal microvascular resistance may be regulated.

Acknowledgments

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