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Possible role of adenosine in macula densa control of glomerular hemodynamics

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Possible role of adenosine in macula densa control of glomerular hemodynamics.

Background. The macula densa (MD), a plaque of specialized tubular epithelial cells, senses changes in tubular NaCl concentration and sends a signal(s) that controls the resistance of the glomerular afferent arteriole (Af-Art). This mechanism, called tubuloglomerular feedback (TGF), is thought to be important in the homeostasis of body fluids and electrolytes. Our aim was to determine the range of NaCl concentrations in tubular fluid at the MD that would elicit the Af-Art response. In addition, we examined the possible involvement of adenosine in transmitting the signal from the MD to the Af-Art.

Methods. Rabbit Af-Arts and attached MD were simultaneously microperfused in vitro, keeping pressure in the Af-Art at 60 mm Hg.

Results. Increasing the Na^+/Cl^- concentration of the MD perfusate from 26/7 to 41/22 mEq/L decreased the luminal diameter of the terminal Af-Art segment by 10 ± 4% (N = 9; $P < 0.01$). The response was maximal at 55/36 mEq/L (18 ± 6%), so that further elevation of NaCl concentration had no additional effect (20 ± 6% at 84/65 mEq/L). When FK838 (10^{-6} mol/L), a specific adenosine A_1 receptor antagonist, was added to both Af-Art perfusate and bath, Af-Art constriction was completely abolished. The maximum response was 20 ± 3% before FK838 and 0.6 ± 1% afterward (N = 12). Adding adenosine at 10^{-8} mol/L to both bath and perfusate significantly augmented Af-Art constriction induced by increased NaCl at the MD ($P < 0.01$); however, adding 10^{-8} to 10^{-6} mol/L adenosine to the MD perfusate had no effect regardless of the NaCl concentration at the MD.

Conclusions. These results demonstrate that MD control of Af-Art resistance is induced by relatively low NaCl concentrations at the MD, and that activation of the adenosine A_1 receptor in the vascular and interstitial space (but not the tubular lumen) may be essential for signal transmission from the MD to the Af-Art.

In each nephron of the mammalian kidney, the tubule returns to the hilus of the parent glomerulus, forming the juxtaglomerular apparatus (JGA) [1]. The JGA displays a unique arrangement of a plaque of specialized tubular epithelial cells, called the macula densa (MD), and the glomerular afferent arteriole (Af-Art) and efferent arteriole (Ef-Art). Micropuncture studies have shown that increased NaCl concentration at the distal tubule lowers the single-nephron glomerular filtration rate (SNGFR), a phenomenon called tubuloglomerular feedback (TGF) [2, 3]. It is thought that the MD senses changes in the composition of tubular fluid and sends a signal(s) that controls Af-Art resistance and hence SNGFR.

It has been proposed that adenosine, generated locally as the result of increased NaCl transport [4, 5], may be important in signal transmission of TGF [6, 7]. Despite its vasodilator action through A_2 receptors in most other vessels (including Ef-Arts), adenosine at physiological concentrations induces constriction of the Af-Art through A_1 receptors (abstract; Ren et al, *J Am Soc Nephrol* 4: 566, 1993) [8]. Studies have shown that infusion of adenosine receptor antagonists into the systemic circulation, peritubular capillaries or loop of Henle attenuates the TGF response [8–10]. Although these studies suggest that adenosine may be involved in transmission of the TGF signal, neither the mechanism nor the site of action is clear.

The purpose of this study was to determine (*1*) the range of NaCl concentrations that induces Af-Art constriction, (*2*) whether adenosine is involved in signal transmission from the MD to the Af-Art, and if so, (*3*) whether it acts in the tubular lumen or vasculature. For this, rabbit Af-Arts were isolated with the glomerulus intact together with adherent tubular segments including the MD, and they were perfused simultaneously. This preparation allows us to observe the Af-Art response directly while controlling the composition of the tubular fluid at the MD.

METHODS

Isolation and microperfusion of the rabbit Af-Art with MD attached

We used methods that were similar to those described previously to isolate and microperfuse Af-Arts with MD attached [11, 12]. Briefly, young male New Zealand white

Key words: afferent arteriole, adenosine A_1 receptor, tubuloglomerular feedback, microperfusion.

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rabbits (1.5 to 2.0 kg), fed standard rabbit chow (Ralston Purina, St. Louis, MO, USA) and given tap water ad libitum, were anesthetized with sodium pentobarbital (40 mg/kg, IV) and given an injection of heparin (500 U, IV). The kidneys were removed and sliced along the corticomedullary axis. Slices were placed in ice-cold minimum essential medium (MEM; Gibco, Grand Island, NY, USA) containing 5% bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) and dissected under a stereomicroscope (SZH; Olympus, Tokyo, Japan) as described previously [11, 12]. From each rabbit, a single superficial Af-Art and its intact glomerulus were microdissected together with adherent tubular segments consisting of portions of the thick ascending limb of the loop of Henle, MD and early distal tubule. Using a micropipette, the microdissected complex was transferred to a temperature-regulated chamber mounted on an inverted microscope (IMT-2; Olympus) with Hoffman modulation. Both the Af-Art and the end of either the distal tubule or thick ascending limb were cannulated with an array of glass pipettes as described previously [11, 12]. Intraluminal pressure was measured by Landis' technique, using a fine pipette introduced into the Af-Art through the perfusion pipette. The Af-Art was perfused with oxygenated MEM (95% O_2 and 5% CO_2) containing 5% BSA, and intraluminal pressure was maintained at 60 mm Hg throughout the experiment. The MD was perfused with a modified Krebs-Ringer bicarbonate buffer (oxygenated to pH 7.4) at a rate of 10 nL/min. The basic composition of the low-NaCl buffer was 25 mmol/L $NaHCO_3$, 0.96 mmol/L NaH_2PO_4, 0.24 mmol/L Na_2HPO_4, 5 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1 mmol/L $CaCl_2$, and 5.5 mmol/L glucose (Na^+, 26 mEq/L; Cl^-, 7 mEq/L). The bath consisted of 100 μL MEM containing 0.1% BSA and was exchanged continuously at a rate of 1 mL/min. Microdissection and cannulation were completed within 90 minutes at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once the temperature was stable, a 30-minute equilibration period was allowed before taking any measurements. Images were displayed at magnifications up to 1980× and recorded with a Sony video system consisting of a camera (DXC-755), monitor (PVM1942Q) and video recorder (EDV-9500). The diameter of the terminal segment of the Af-Art (the most responsive segment) was measured with an image-analysis system (Fryer, Carpentersville, IL, USA).

Experimental protocols

Luminal diameter of Af-Arts with various NaCl concentrations at the MD. After the 30-minute equilibration period, Na^+/Cl^- concentrations of the MD perfusate (in mEq/L) were increased from 26/7 to 41/22, 55/36, 84/65, 113/94 and 141/122. The solutions were prepared from a modified Krebs-Ringer solution by eliminating NaCl without adjusting osmolality. The luminal diameter of the Af-Art was observed for at least five minutes at each concentration.

Adenosine-induced Af-Art constriction and its blockade with a selective adenosine A_1 receptor antagonist. After the equilibration period, increasing concentrations of adenosine (10^{-9} to 10^{-6} mol/L) were added to the bath, and luminal diameter was monitored for ten minutes at each dose. In order to study whether adenosine-induced Af-Art constriction is mediated by the adenosine A_1 receptor, the same experiments were repeated in the presence of 6-oxo-3-[2-phenylpyrazolo [1,5-a] pyridine-3-yl]-1(6H)-pyridazinebutyric acid (FK838), a selective non-xanthine antagonist of adenosine A_1 receptors (abstract; Katsunoki et al, *Can J Physiol Pharmacol* 72:505, 1994) [13, 14]. FK838 at 10^{-6} mol/L was added to the bath after the equilibration period and throughout the experiment. FK838 (1 mol/L) was prepared with sodium carbonate (0.27 mmol/L) and diluted with the bath solution. We confirmed that the vehicle had no effect on adenosine-induced Af-Art constriction.

Effect of adenosine A_1 receptor blockade on Af-Art constriction induced by high NaCl at the MD. To study whether adenosine is involved in the MD control of Af-Art resistance, we examined the maximum response of Af-Arts to increased NaCl concentrations at the MD before and after blocking the adenosine A_1 receptors. The MD was first perfused with the low Na^+/Cl^- solution (26/7 mEq/L) for five minutes, and then the perfusate was changed to the high Na^+/Cl^- solution (55/36 or 84/65 mEq/L) that elicited the maximal response (**Results** section). Five minutes later, the MD perfusate was again changed to the low-NaCl solution, and then FK838 (10^{-6} mol/L) was added to the bath and arteriolar perfusate. Following a 20-minute equilibration period, the MD was again perfused with the high-NaCl solution. To confirm the reproducibility of the Af-Art responses, time control experiments were performed as described above, except that vehicle was used instead of FK838.

Effect of vascular and interstitial adenosine on Af-Art constriction induced by high NaCl at the MD. Blocking the adenosine A_1 receptors with FK838 completely abolished the Af-Art constriction induced by high NaCl at the MD (**Results** section). We next examined whether increasing adenosine levels in the juxtaglomerular interstitium and Af-Art lumen may affect the Af-Art response to increased NaCl concentrations at the MD. The Af-Art diameter was measured when the MD was not perfused, perfused with the low-NaCl solution (26/7 mEq/L), and perfused with the moderate-NaCl solution (41/22 mEq/L). MD perfusion was then stopped and 10^{-8} mol/L adenosine was added to both bath and Af-Art perfusate. After an additional 20-minute equilibration period, the MD was perfused with solutions having the same composition as above.

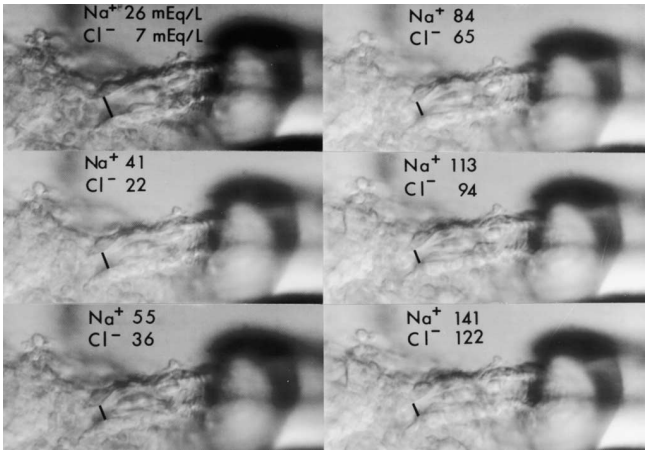

Fig. 1. Afferent arteriole (Af-Art) response to increased tubular NaCl concentrations at the macula densa (MD).

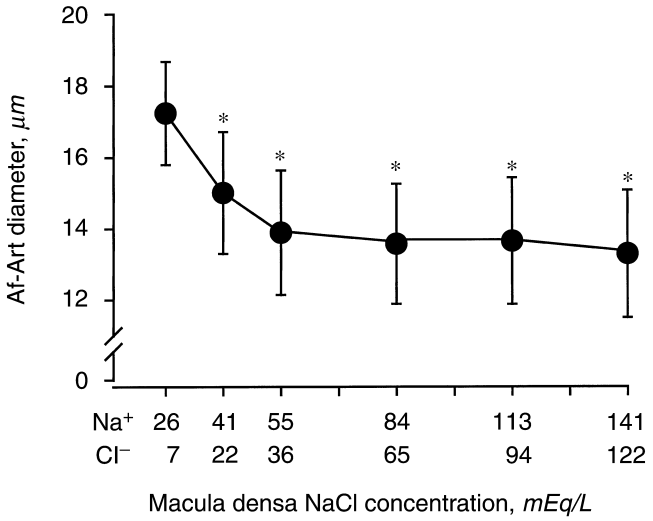

Fig. 2. Effect of adenosine on the afferent areteriole (Af-Art) diameter before and after blocking the adenosine A_1 receptor. *$P < 0.007$ compared with basal diameter. Adenosine caused dose-dependent vasoconstriction, and FK838 completely blocked the Af-Art constriction induced by adenosine.

Effect of tubular adenosine on Af-Art diameter with various concentrations of NaCl at the MD. To examine whether increasing adenosine levels in the tubular lumen causes Af-Art constriction, the MD was perfused with a solution containing Na^+/Cl^- at either 26/7 or 141/122 mEq/L throughout the experiment. Increasing concentrations of adenosine (10^{-8} to 10^{-6} mol/L) were added to the MD perfusate, and Af-Art luminal diameter was observed for ten minutes at each dose.

Statistics

Values were expressed as mean ± SEM. The Student paired *t* test was used to examine whether the diameter at a given concentration was different from the control value. Analysis of covariance (ANCOVA) was used to examine whether the change in diameter at a given concentration was different between groups. For both analyses, $P < 0.05$ was considered significant.

RESULTS

Luminal diameter of Af-Arts with various NaCl concentrations at the MD

Figure 1 illustrates the Af-Art response to increased NaCl concentration, while Figure 2 summarizes the results. Increasing NaCl concentration (Na^+/Cl^-) by as little as 15 mEq/L (from 26/7 to 41/22 mEq/L) significantly decreased Af-Art diameter from 17.2 ± 1.4 to 15.0 ± 1.7 μm ($N = 9$; $P < 0.01$). When Na^+/Cl^- reached 55/36 mEq/L, the response was already maximum (with diameter decreasing to 13.9 ± 1.8 μm), and no further constriction was observed at higher concentrations. Thus, a full response was completed with a change in NaCl concentration of as little as 30 mEq/L. Constriction was strongest at the terminal segment of the Af-Art (Fig. 1).

Adenosine-induced Af-Art constriction and its blockade with a selective adenosine A_1 receptor antagonist

Basal luminal diameter was 18.1 ± 0.8 μm ($N = 13$) and was not altered by adenosine until the concentration reached 10^{-7} mol/L. However, at 10^{-7} mol/L and 10^{-6} mol/L, the diameter decreased significantly to 15.0 ± 0.8 and 11.8 ± 0.8 μm, respectively ($P < 0.007$; Fig. 3). Adenosine-induced constriction was observed within a few seconds after adding adenosine, and it remained stable for at least ten minutes. FK838 at 10^{-6} mol/L did not alter basal diameter (19.7 ± 2.9 vs. 19.7 ± 2.7 μm before and after FK838, respectively); however, it blocked the Af-Art constriction induced by adenosine up to 10^{-6} mol/L (Fig. 3).

Effect of adenosine A_1 receptor blockade on Af-Art constriction induced by high NaCl at the MD

Figure 4 illustrates the Af-Art response induced by changing the MD perfusate from low to high NaCl before and after blocking A_1 receptors with FK838, while results are summarized in Figure 5. Since solutions containing Na^+/Cl^- at either 55/36 or 84/65 mEq/L induced maximum and indistinguishable responses, the results were combined and analyzed collectively. When NaCl concentration was increased from low to high, luminal diameter decreased by 20 ± 3%, from 18.1 ± 0.8 to 14.3 ± 0.9 μm ($N = 12$; $P < 0.001$). Treatment with FK838 had no effect on basal diameter (18.0 ± 0.9 μm), but completely blocked the constriction induced by high NaCl at the MD (0.6 ± 1%), which time control experiments showed was reproducible.

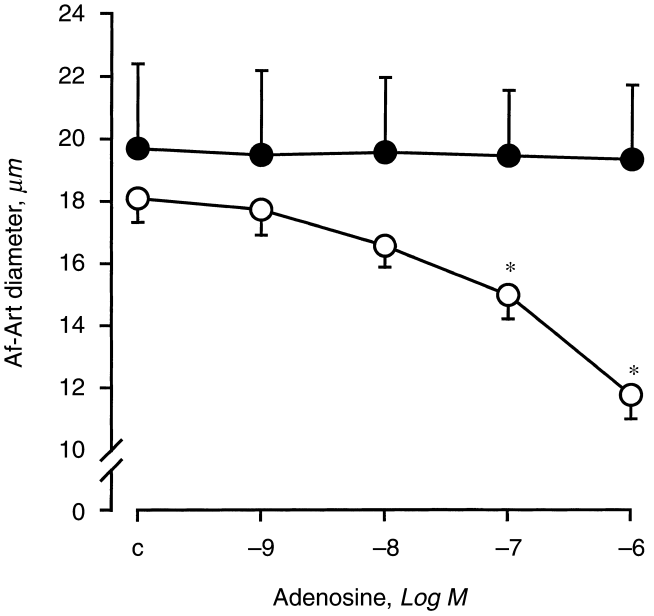

Fig. 3. Effect of various NaCl concentrations in the macula densa perfusate on Af-Art luminal diameter. $*P < 0.01$ compared with basal diameter. Increasing the NaCl concentration (Na^+/Cl^-) by as little as 15 mEq/L (from 26/7 to 41/22 mEq/L) significantly decreased Af-Art diameter. When Na^+/Cl^- reached 55/36 mEq/L, the response was already maximum and no further constriction was observed with higher NaCl concentrations. Symbols are: (●) FK838 10^{-6} mol/L, $N = 5$; (○) nontreated, $N = 13$.

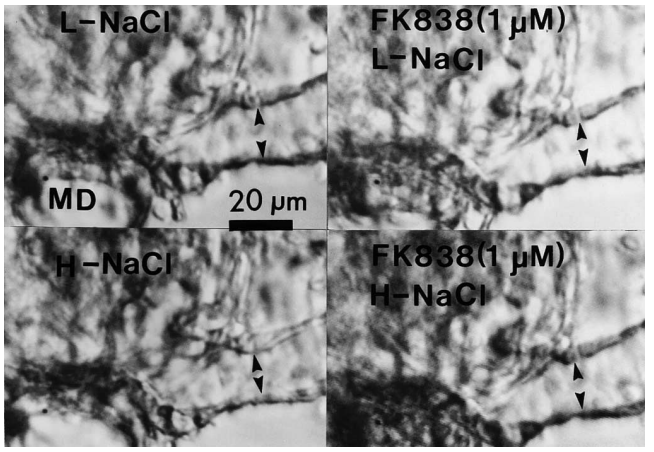

Fig. 4. Af-Art response induced by changing the macula densa (MD) perfusate from low (L) to high (H) NaCl before and after blocking the adenosine A_1 receptor. Abbreviations are: FK838, adenosine A_1 receptor antagonist.

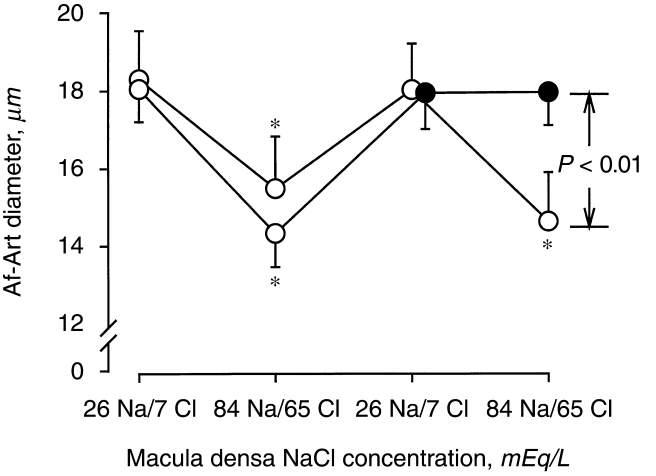

Fig. 5. Effect of FK838 on Af-Art constriction induced by high NaCl at the macula densa. $*P < 0.01$, low vs. high NaCl concentration in the macula densa perfusate. Symbols are: (○) non-treated arterioles ($N = 8$); (●) FK838-pretreated arterioles ($N = 12$). When NaCl concentration was increased from low to high, Af-Art luminal diameter decreased by 20 ± 3%. Treatment with FK838 had no effect on basal diameter but completely blocked the constriction induced by high NaCl at the macula densa. Time control experiments demonstrated that this constriction was reproducible.

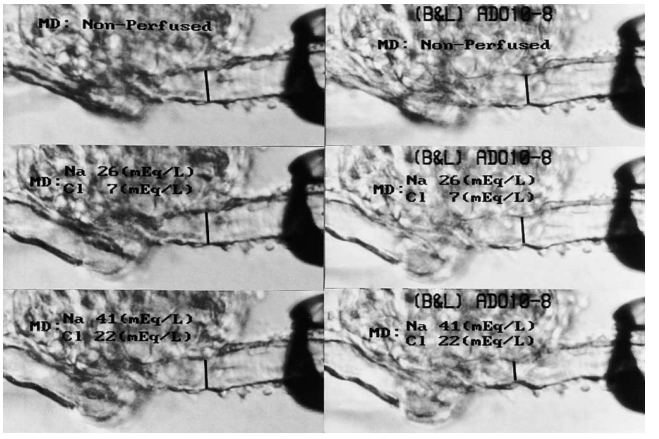

Fig. 6. Augmented Af-Art constriction induced by high NaCl at the macula densa (MD) in the presence of vascular and interstitial adenosine at 10^{-8} mol/L. Abbreviations are: B, bath; L, arteriolar perfusate; ADO, adenosine.

Effect of vascular and interstitial adenosine on Af-Art constriction induced by high NaCl at the MD

Figure 6 illustrates the Af-Art response to MD perfusion with solutions containing Na^+/Cl^- at either 26/7 or 41/22 mEq/L before and after adding adenosine to the Af-Art lumen and bath, while results are summarized in Figure 7. Basal diameter of Af-Arts without MD perfusion was 15.6 ± 1.5 μm ($N = 9$). Perfusion of the MD with solutions containing Na^+/Cl^- at 26/7 mEq/L had no effect on diameter (15.7 ± 1.6 μm), while increasing Na^+/Cl^- concentration to 41/22 mEq/L decreased diameter by 10 ± 4% to 14.1 ± 1.5 μm. Adenosine added to both Af-Art perfusate and bath at a concentration of 10^{-8} mol/L had no effect on luminal diameter when the MD was not perfused (15.3 ± 1.4 μm); however, as soon as the MD was perfused with low NaCl, the Af-Art constricted significantly by 18 ± 6% to 12.7 ± 1.7 μm, and when Na^+/Cl^- concentration was increased to 41/22

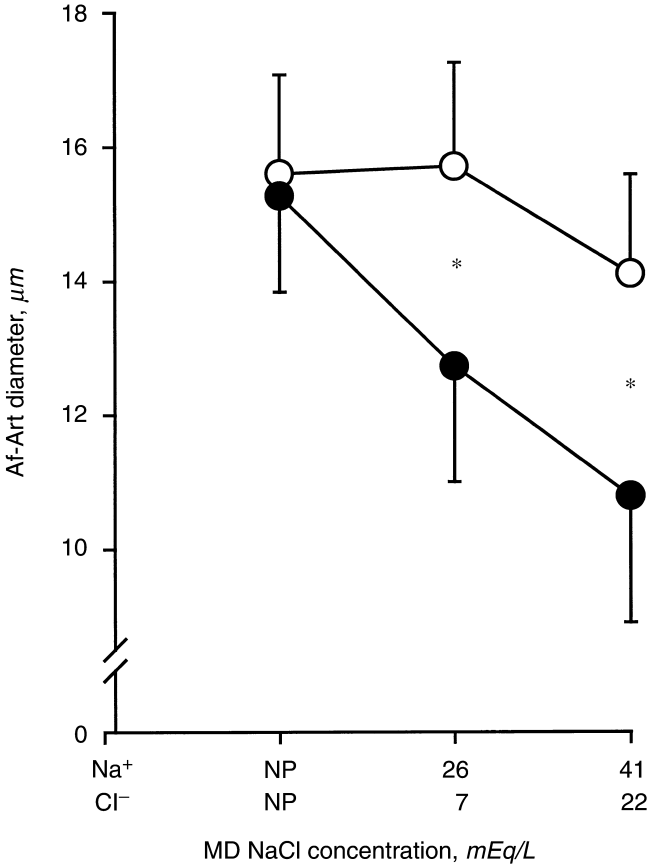

Fig. 7. Effect of vascular and interstitial adenosine on Af-Art responses to increased NaCl at the macula densa. $*P < 0.02$ before *vs.* after adding adenosine (10^{-8} mol/L) to the arteriolar perfusate and bath. Symbols are: (○) before adding adenosine; (●) after adding adenosine ($N = 9$). Abbreviations are: Af-Art, afferent arteriole; NP, non-perfused. Perfusion of the macula densa with a solution containing Na^+/Cl^- at 26/7 mEq/L had no effect on diameter, while increasing Na^+/Cl^- concentration to 41/22 mEq/L decreased diameter by 10 ± 4%. Adenosine (10^{-8} mol/L) added to both the arteriolar perfusate and bath had no effect on luminal diameter when the macula densa was not perfused. However, as soon as the macula densa was perfused with low NaCl, the Af-Art constricted significantly by 18 ± 6%, and when the Na^+/Cl^- concentration was increased to 41/22 mEq/L the diameter decreased by as much as 32 ± 8%. This decrease was significantly greater when adenosine was present in the interstitial space.

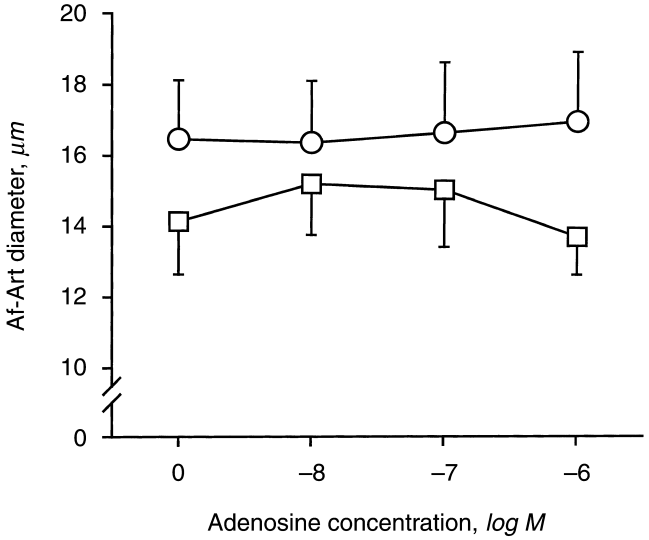

Fig. 8. Effect of tubular adenosine on afferent arteriole (Af-Art) responses to various concentrations of NaCl in the macula densa (MD) perfusate. Symbols are: (○) 26/7 mEq/L Na^+/Cl^- concentration ($N = 5$); (□) 141/122 mEq/L Na^+/Cl^- concentration ($N = 4$). Adding increasing concentrations of adenosine to the MD perfusate had no effect on Af-Art diameter regardless of the NaCl concentration.

mEq/L the diameter decreased by as much as 32 ± 8% (to 10.8 ± 1.9 μm). The decrease in diameter was significantly greater in the presence of vascular and interstitial adenosine ($P < 0.01$).

Effect of tubular adenosine on Af-Art diameter with various concentrations of NaCl at the MD

As shown in Figure 8, adding increasing concentrations of adenosine to the MD perfusate had no effect on Af-Art diameter regardless of NaCl concentration ($N = 4$ to 6).

DISCUSSION

The MD senses NaCl concentrations in tubular fluid and controls Af-Art resistance. In this process, called TGF, increased NaCl concentration at the MD constricts the Af-Art, thereby decreasing glomerular capillary pressure and SNGFR [2, 3]. Holstein-Rathlou and Marsh [15] have simultaneously measured both Cl^- concentration of early distal tubular fluid and pressure in the proximal tubule (as an index of SNGFR) and found synchronous oscillation at a frequency of about 2 cycles per minute. Interestingly, pressure started to decline only a couple of seconds after Cl^- concentration started to rise, that is, only a small change in Cl^- concentration was needed to elicit a pressure response. Consistent with this finding, our results demonstrate that MD control of Af-Art resistance was extremely sensitive, attaining a complete response with a change in NaCl concentration of as little as 30 mEq/L (from 26/7 to 55/36 mEq/L Na^+/Cl^-). The maximal response was reached at a NaCl concentration of 55/36 mEq/L, which is close to the micropuncture data [16]. Since the physiological range of NaCl concentration at the MD is reportedly between 20 and 80 mEq/L [17], our results indicate that a small change in NaCl concentration at the MD, within the physiological range, has a profound influence on SNGFR.

Although nearly 40 years have passed since the discovery of the TGF response [3], the mechanism(s) by which the MD transforms the luminal signal (NaCl concentration in the tubular fluid) into the Af-Art response remains unresolved. However, accumulating experimental evidence supports an important role of adenosine in the signaling mechanism of TGF [6, 7]. In addition, evidence suggests that adenosine is involved in another important

function of the MD, control of renin release from the juxtaglomerular cells [6]. It has been demonstrated that the level of intrarenal adenosine increases during acute [7] or chronic sodium loading of the kidney [18] and that adenosine constricts the Af-Art but dilates most other vessels, including the Ef-Art (abstract; Ren et al, *J Am Soc Nephrol* 4:566, 1993) [8, 19]. Thus, it may be hypothesized that in response to increased tubular NaCl, the MD produces adenosine, which in turn elicits Af-Art constriction. Consistent with this hypothesis, micropuncture studies have demonstrated that administration of adenosine receptor antagonists (nonspecific or specific adenosine A_1 receptor antagonists) or dipyridamole (which elevates extracellular adenosine levels by inhibiting cellular adenosine uptake) attenuates and potentiates the TGF response, respectively [8–10, 20]. However, these in vivo studies did not completely eliminate neurohormonal influences, nor did they rule out the possibility that adenosine receptors elsewhere than the JGA might influence the results. For instance, adenosine has been shown to inhibit tubular transport in the thick ascending limb of the loop of Henle [21]. Thus, adenosine antagonists would stimulate tubular transport in this segment, thereby reducing the NaCl concentration of the tubular fluid reaching the MD. Such tubular actions may contribute indirectly to attenuation of the TGF response by adenosine receptor antagonists. To examine the role of adenosine in the MD control of Af-Art resistance directly, we employed an in vitro preparation of isolated JGAs and found that blocking the adenosine A_1 receptor with FK838 abolished the Af-Art constriction induced by increased NaCl at the MD. Taken together with the previous micropuncture studies, our results suggest that activation of the adenosine A_1 receptor is essential for signal transmission from the MD to the Af-Art within the JGA.

The site of adenosine action involved in the TGF response remains unclear. Franco et al reported that intraluminal administration of 1,3-dipropyl-8-sulfophenylxanthine (a nonspecific adenosine A_1 and A_2 antagonist) to the thick ascending limb of Henle's loop completely abolished the TGF response, whereas systemic administration had no effect, suggesting that adenosine receptors present in the tubular rather than the vascular compartment are involved in TGF [9]. In contrast, Osswald et al reported that systemic administration of theophylline (a nonspecific adenosine A_1 and A_2 antagonist) attenuated the TGF response [8]. Schnermann et al have shown that suffusing the peritubular capillaries with 8-cyclopentyl-1,3-dipropylxantin (DCPCX), a widely used xanthine-derived adenosine A_1 receptor antagonist, markedly attenuated the decrease in stop-flow pressure induced by loop perfusion, suggesting that vascular A_1 receptors in the JGA play an important role in the TGF response [10]. The reason for these discrepancies is not clear, but may be related to differing pharmacokinetics of the adenosine antagonists used and/or systemic neurohormonal influences. To examine directly whether adenosine acts in the tubular lumen or interstitium, adenosine was added to the MD perfusate or to both the bath and Af-Art perfusate of the isolated JGA preparation. Adding adenosine to the interstitium (bath and Af-Art perfusate) at a concentration that had no direct effect on basal Af-Art diameter (10^{-8} mol/L) significantly augmented the Af-Art constriction induced by high NaCl at the MD, whereas adding adenosine to the MD perfusate had no effect. Thus, our results strongly support the hypothesis that adenosine in the vascular and interstitial space but not the tubular lumen plays an important role in signal transmission from the MD to the Af-Art. This concept is supported by the finding that DCPCX inhibited TGF responses more when it was added to the lumen of neighboring nephrons than to the test nephron [10]. In addition, Traynor et al have recently demonstrated that adding an adenosine A_1 agonist to the perfusate of Henle's loop constricted the Af-Art of the neighboring nephron [22], suggesting that vasoconstriction is mediated through extratubular (presumably vascular) rather than luminal A_1 receptors. Therefore, it may be possible that adenosine generated by MD cells in response to increased NaCl transport is delivered across the basolateral membrane, where it gains access to the juxtaglomerular interstitium and binds to A_1 receptors on the vascular smooth muscle cells of Af-Arts or extraglomerular mesangial cells. Indeed, expression of an AMP-specific 5′-nucleotidase has been demonstrated in MD cells (abstract; Walker et al, *FASEB J*, 9:A843, 1995), and a high density of adenosine A_1 receptors in the terminal segment of Af-Arts has been shown by in situ hybridization of A_1 receptor mRNA and by functional studies of isolated perfused Af-Arts [23, 24]. However, our study does not prove that the MD produces adenosine in response to increased NaCl delivery, nor does it clarify whether adenosine is the mediator or a modulator of TGF. It is possible that activation of the adenosine A_1 receptor is necessary in order for the TGF-dependent vasoconstrictor signals to be effective.

Since adenosine is generated as a consequence of adenosine triphosphate (ATP) hydrolysis, a precursor of adenosine such as ATP may be the actual substance released by the MD in response to increased NaCl delivery. It has been reported that MD cells are richly endowed with small mitochondria distributed along the basal and lateral aspects [25, 26] and exhibit relatively low Na^+,K^+-ATPase activity [27], supporting the concept that MD cells possess substantial ATP-generating capacity. In addition, Mitchell and Navar demonstrated that infusion of ATP into the peritubular capillaries decreased proximal tubule stop-flow pressure, suggesting that ATP causes preglomerular vasoconstriction [28].

Taken together with our findings, it may be that increased NaCl delivery to the MD increases the rate of ATP utilization, leading to increased formation of adenosine, which then diffuses to the interstitium where it constricts Af-Arts acting via A_1 receptors. However, this hypothesis is inconsistent with several studies demonstrating that ATP directly constricts Af-Arts (but not through its conversion to adenosine, because adenosine receptor antagonists do not attenuate ATP-induced constriction) [24, 29, 30]. Further studies examining the mechanism(s) that stimulate adenosine formation in response to increased NaCl delivery to the MD and clarifying the mechanism(s) of signal transmission from the MD to the Af-Art will provide valuable new insight into the TGF response.

One may ask why TGF was significantly augmented by adding a low concentration of adenosine (10^{-8} mol/L) to the bath and Af-Art lumen, which had no direct effect on basal Af-Art diameter. While the mechanism(s) involved is not clear, there are two possibilities. First, as discussed above, increased NaCl delivery to the MD leads to increased endogenous adenosine formation, which when combined with exogenous adenosine significantly increases Af-Art constriction. Second, pretreatment with a low concentration of adenosine may compete for adenosine deaminase, thereby decreasing endogenous adenosine hydrolysis and increasing the Af-Art response to endogenous adenosine.

The present study design used FK838, a non-xanthine type adenosine A_1 antagonist, to block the adenosine A_1 receptor. FK838 reportedly exhibits a very high selectivity for A_1 receptors, with a >650-fold affinity compared to A_2 receptor (abstract; Katsunoki et al, *Can J Physiol Pharmacol* 72:505, 1994) [13, 14]. We also showed that FK838 blocks adenosine-induced constriction (mediated by A_1 receptors) in the Af-Art, but has no effect on adenosine-induced dilatation (mediated by A_2 receptors) in the Ef-Art (abstract; Ren et al, *J Am Soc Nephrol* 4:566, 1993). Such effects of FK838 did not differ from those of DCPCX. In addition, we confirmed that FK838 had no effect on the vasoconstrictor action of norepinephrine in Af-Arts. Thus, the action of FK838 in blocking Af-Art constriction induced by high tubular NaCl at the MD was likely due to A_1 receptor blockade, but not a nonspecific action of FK838.

In summary, we found that increasing NaCl concentration at the MD by as little as 30 mEq/L (Na^+/Cl^- from 26/7 to 55/36 mEq/L) caused maximum constriction of Af-Arts, and this TGF-mediated constriction of Af-Arts was abolished when the adenosine A_1 receptor was blocked with FK838. Addition of exogenous adenosine to the vascular and interstitial space but not the tubular lumen significantly augmented the Af-Art constriction induced by high NaCl at the MD. These results provide evidence that MD control of Af-Art resistance is very sensitive within the physiological range of NaCl concentrations at the MD, and that activation of the adenosine A_1 receptor in the vascular and interstitial space but not the tubular lumen is essential for signal transmission from the MD to the Af-Art.

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