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Control of rat glomerular microcirculation by juxtaglomerular adenosine A1 receptors

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Control of rat glomerular microcirculation by juxtaglomerular adenosine A1 receptors. The role of adenosine A1 receptors in the glomerular microcirculation and tubuloglomerular feedback (TGF) was studied in anesthetized Sprague-Dawley rats. TGF activity was assessed as the reduction in proximal tubular stop-flow pressure (SFP) on establishing orthograde perfusion of the loop of Henle with artificial tubular fluid at 40 nl/min. Administration of a selective A1 receptor antagonist, KW-3902 (0.5 μg/kg/min i.v.), increased fractional excretion of Na (FE_{Na}) 4.3-fold without changing blood pressure, glomerular filtration rate, renal plasma flow, or filtration fraction. SFP in the absence of distal flow (SFP_0) increased, and TGF-mediated SFP reduction was suppressed dose dependently [by 23 ± 2% from an SFP_0 of 34 ± 1 mm Hg, by 15 ± 4% from 36 ± 2 mm Hg, and by 2 ± 1% from 39 ± 1 mm Hg during vehicle, low- and high-dose infusions (0.5 and 5.0 μg/kg/min), respectively]. Intratubular or peritubular capillary administration of 10^{-4} M KW-3902 completely suppressed TGF without affecting SFP_0. TGF suppression and elevation of SFP_0 during systemic A1 blockade indicated vasodilation, both in the afferent arteriole and more proximal preglomerular vessels. Inhibition of tubular Na reabsorption combined with TGF suppression allowed the marked natriuresis. TGF suppression through systemic, luminal, and peritubular application of the drug suggest that juxtaglomerular apparatus A1 receptors are important in the control of glomerular microcirculation.

Glomerular hemodynamics are controlled by tubuloglomerular feedback (TGF), which alters blood flow and glomerular filtration rate (GFR) in a single nephron by changing afferent arteriolar resistance in response to the [NaCl] at the macula densa (MD). Endogenous vasoactive substances contribute to the regulation of afferent arteriolar tone and thus modulate TGF responsiveness [1, 2]. Adenosine, endogenously produced as a metabolite of adenosine triphosphate, is held to be a modulator of renal hemodynamics and tubular function and a candidate for TGF mediation. Adenosine A1 receptors exist in the juxtaglomerular apparatus (JGA) [3]. We investigated the role of adenosine A1 receptor in glomerular microcirculation and TGF in anesthetized rats.

Key words: adenosine receptor, macula densa, natriuresis, tubuloglomerular feedback

METHODS

Male Sprague-Dawley rats kept on commercial chow were anesthetized (thiopental sodium, Ravonal, 110 mg/kg i.p.) and prepared as previously described [4]. Polyfructosan (10% in 0.9% saline; Inutest, Laevosan, Austria) was infused i.v. at 4.5 ml/kg/hr. Arterial pressure was monitored and blood sampled through the right femoral artery. The left kidney was prepared for micropuncture, and the ureter was cannulated at the pelvis to collect urine samples. A 25-gauge needle connected to a PE-50 tube was inserted into the left renal vein to collect renal venous blood. Clearances were measured during i.v. administration of a specific adenosine A1 receptor antagonist, KW-3902 [5], at 0.5 μg/kg/min.

The TGF response was assessed as the change in proximal stop-flow pressure (SFP), an index of intraglomerular pressure (P_{gc}), on establishing orthograde perfusion of the loop of Henle with artificial tubular fluid (ATF) from the last accessible proximal segment at 40 nl/min. The response was assessed during i.v. infusion of KW-3902 at 0.5 or 5.0 μg/kg/min; during loop perfusion at 40 nl/min with 10^{-4} M KW-3902 in ATF and during peritubular capillary microinfusion of 10^{-4} M KW-3902 in 0.9% saline at 20 nl/min.

Glomerular filtration rate, renal plasma flow (RPF), and renal blood flow were calculated using standard formulas and renal vascular resistance as mean blood pressure/renal blood flow. Results are expressed as mean ± SEM. Student's paired or unpaired *t*-test or analysis of variance was used for comparison. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

KW-3902 (0.5 μg/kg/min i.v.) increased urine flow by 38%, fractional excretion of Na (FE_{Na}) 4.3-fold, and FE_K by 34% without changing mean blood pressure, GFR, RPF, or filtration fraction. During vehicle infusion, SFP in the absence of distal flow (SFP_0) was 34 ± 1 mm Hg and fell on loop perfusion by 23 ± 2%. Systemic KW-3902 at low and

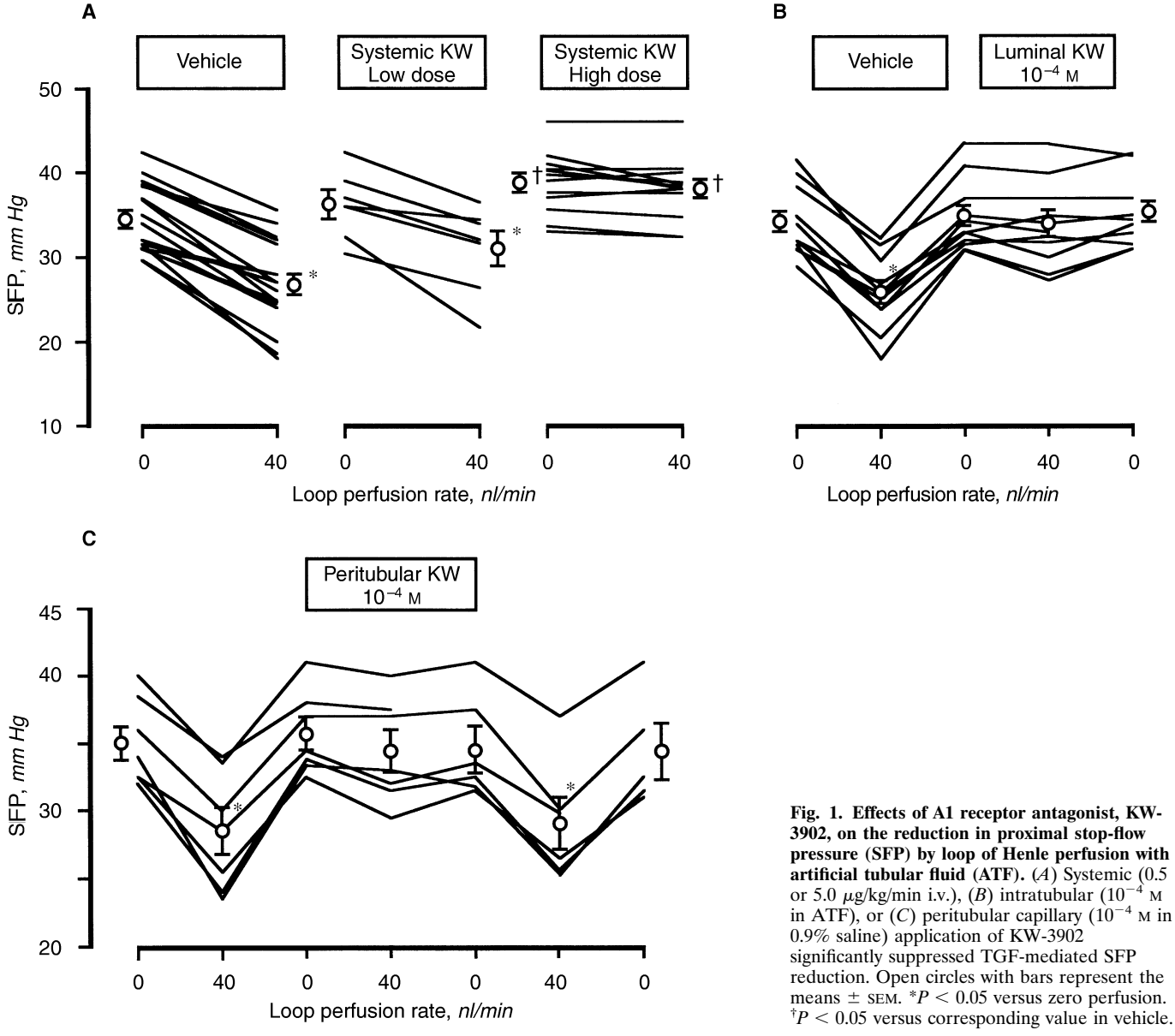

Fig. 1. Effects of A1 receptor antagonist, KW-3902, on the reduction in proximal stop-flow pressure (SFP) by loop of Henle perfusion with artificial tubular fluid (ATF). (*A*) Systemic (0.5 or 5.0 μg/kg/min i.v.), (*B*) intratubular (10^{-4} M in ATF), or (*C*) peritubular capillary (10^{-4} M in 0.9% saline) application of KW-3902 significantly suppressed TGF-mediated SFP reduction. Open circles with bars represent the means ± SEM. $^{*}P < 0.05$ versus zero perfusion. $^{\dagger}P < 0.05$ versus corresponding value in vehicle.

high doses (0.5 and 5.0 μg/kg/min) increased SFP_0 to 36 ± 2 and 39 ± 1 mm Hg and suppressed TGF-mediated SFP reduction to 15 ± 4% and 2 ± 1%, respectively. Intraluminal KW-3902 completely suppressed the TGF response: SFP remained unchanged from 35 ± 1 to 34 ± 2 mm Hg during loop perfusion with 10^{-4} M KW-3902 in ATF, whereas SFP decreased from 34 ± 1 to 26 ± 1 mm Hg during vehicle application. Microinfusion of 10^{-4} M KW-3902 at 20 nl/min into peritubular capillary of the tested nephron suppressed the SFP response to loop perfusion; SFP remained unchanged from 36 ± 1 to 34 ± 2 mm Hg, whereas SFP was reduced from 35 ± 1 to 28 ± 2 mm Hg before KW-3902 infusion and from 35 ± 2 to 29 ± 2 mm Hg in the recovery period.

Our findings show that intravenous, intraluminal, and peritubular administration of a selective A1 receptor antagonist, KW-3902, decreases the maximal reduction in SFP induced by loop perfusion, indicating attenuation of TGF response. Because the TGF response involves the vasomotor response of the afferent arteriole, the blunted response of TGF suggests dilation of this arteriolar segment by KW-3902. This is in accordance with results from *in vitro* isolated arterioles of rabbit kidneys in which afferent arteriolar constriction induced by an adenosine analog is reversed completely by coadministration of a selective A1 antagonist [6]. On the other hand, i.v. administration of adenosine receptor antagonists does not affect the TGF response [7, 8], whereas intraluminal administration clearly inhibits TGF [7]. The discrepancy between these results and this study may be due to the low selectivity

for the A1 receptor of the agent used [7] or volume replacement by plasma [8], which is known to attenuate the TGF response.

In this study, A1 receptor blockade with KW-3902, via peritubular capillary or tubular lumen, suppressed TGF-mediated SFP reduction but did not alter SFP_0 (that is, in the absence of TGF control). This indicates that A1 blockade via these routes does not affect the basal tone of the afferent arterioles and that the effect of A1 blockade becomes evident only when TGF mechanism is stimulated. Because these two routes of application deliver the agent through capillary wall or tubular cells into the interstitium surrounding the glomerular vessels, thus avoiding systemic effects, our results suggest that endogenous adenosine and its A1 receptor activation in the JGA serve as the communication link between MD cells and afferent arterioles to activate the TGF mechanism.

In addition to TGF suppression, systemic A1 blockade elevated SFP, implying an increase in P_{gc} in the absence of TGF control. This suggests vasodilation of proximal preglomerular vessels in addition to the afferent arteriole in which, at the entrance into the glomerulus, the TGF-mediated vascular response occurs. Furthermore, systemic A1 receptor antagonism stimulates the renin-angiotensin system, which may elevate P_{gc} through the vasoconstriction of efferent arterioles [8].

Intravenous KW-3902 induced a marked natriuresis without affecting any whole-kidney hemodynamic parameters, indicating the inhibition of sodium reabsorption in tubular site(s). A1 receptor mRNA is found widely along nephron segments [9], and A1 receptor-mediated tubular effects have been shown in *in vitro* preparations of the proximal convoluted tubule using a selective A1 antagonist, FK453 [10]. However, inhibition of proximal reabsorption alone would result in the increase in fluid delivery into the loop of Henle. This, through the activation of TGF, decreases GFR and thereby attenuates the natriuresis. In this study, TGF-mediated SFP reduction was suppressed, and SFP without loop perfusion dose dependently increased. Inhibition of tubular sodium reabsorption combined with the effects on glomerular microcirculation is suggested to be the mechanism of the marked natriuresis by KW-3902. In addition, these findings agree with observations that intravenous infusion of a nonxanthine, selective A1 antagonist, FK838, suppresses TGF-mediated reduction in early proximal flow rate, an index of single nephron GFR, and also induced natriuresis without affecting GFR or RPF [11], suggesting a common mechanism in natriuresis through systemic A1 receptor antagonism.

In conclusion, a selective A1 antagonist KW-3902 antagonizes MD-mediated afferent arteriolar vasoconstriction and thus suppresses TGF. TGF suppression through systemic, luminal, and peritubular application of the drug suggests that the activation of A1 receptor in JGA by endogenous adenosine plays an important role in the control of glomerular microcirculation. The marked natriuresis, induced by direct effects on tubular A1 receptors, combined with effects on intrarenal arterioles, implies a role for A1 receptors in renal sodium handling.

APPENDIX

Abbreviations used in this article are: ATF, artificial tubular fluid; FE_{Na}, fractional excretion of Na; GFR, glomerular filtration rate; JGA, juxtaglomerular apparatus; MD, macula densa; RPF, renal plasma flow; SFP, stop-flow pressure; TGF, tubuloglomerular feedback.

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