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The attenuated effect of ATP-sensitive K^+ channel opener pinacidil on renal haemodynamics in spontaneously hypertensive rats

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Abstract

In hypertension, impairment of hyperpolarization by K^+ efflux through ATP-sensitive K^+ (K_{ATP}) channels may contribute to the elevated renal vascular resistance. To elucidate such a role for K_{ATP} channels in the renal vasculature, we used micropuncture techniques to examine the effect of K_{ATP} channel opener, pinacidil (0.15 mg/h per kg body wt i.v.), on renal and glomerular haemodynamics in spontaneously hypertensive rats (SHR) and in normotensive controls (Wistar Kyoto, WKY). Since pinacidil reduced blood pressure significantly in both groups, the abdominal aorta was clamped before pinacidil administration to yield a renal perfusion pressure equivalent to that during pinacidil infusion. Pinacidil significantly decreased renal vascular resistance in both groups, but the relative change from baseline value was greater in WKY than in SHR. These effects of pinacidil were abolished by pretreatment with glibenclamide (3 mg/kg body wt i.v.). Proximal tubular stop-flow pressure (P_{sf}), an index of glomerular capillary pressure, was significantly elevated by pinacidil infusion in WKY, a response abolished by pretreatment with glibenclamide, but not in SHR. The tubuloglomerular feedback response of P_{sf} was not affected by pinacidil in either group. These data suggest that the activity of K_{ATP} channels in SHR may be attenuated in the renal microvasculature. This may contribute to the elevated vascular tone in the renal preglomerular vasculature in SHR.

Keywords: K^+ channel; Hypertension; Tubuloglomerular feedback; Renal haemodynamics; Stop-flow pressure

1. Introduction

Different types of K^+ channel are involved in the fine tuning of the permeability of the cell membrane for K^+, which is a major determinant of cell membrane potential. ATP-sensitive K^+ (K_{ATP}) channels are inhibited by physiological intracellular ATP concentration ($[ATP]_i$) and are thus mostly closed during resting conditions but open as $[ATP]_i$ rises in hypoxic conditions during reduced tissue perfusion (Nelson and Quayle, 1995). However, these channels may also be regulated by receptor activation through the action of autacoids released from non-smooth muscle cells (Nelson and Quayle, 1995).

Physiological or pathophysiological roles of K_{ATP} channels in renal haemodynamics are not well-elucidated. Hypoxia-induced vasodilatation by activation of K_{ATP} channels can impair the myogenic response of the renal vasculature (Loutzenhiser and Parker, 1994). In vitro investigations on renal microvessels have revealed significant effects of K_{ATP} channel openers in dilating afferent (Lorenz et al., 1992; Reslerova and Loutzenhiser, 1995) or efferent arterioles (Reslerova and Loutzenhiser, 1995). However, there are only few data on the effect of K_{ATP} channel openers in the regulatory mechanisms of the renal microcirculation in vivo. Furthermore, K_{ATP} channel activity may be attenuated in hypertension (Ohya et al., 1996), possibly resulting in elevation of the renal vascular resistance, a finding made consistently in hypertension and possibly contributing to the pathogenesis of hypertension.

To elucidate the role of K_{ATP} channels in the regulation of renal vascular function in hypertension, we performed micropuncture experiments in spontaneously hypertensive rats (SHR) and their normotensive controls, Wistar–Kyoto rats (WKY) and explored the effect of the K_{ATP} channel opener pinacidil on glomerular haemodynamics.

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2. Materials and methods

2.1. Animal preparation

Adult (10–12 weeks old) WKY rats and SHR were obtained from Sankyo Labo (Tokyo, Japan). Rats were housed with free access to tap water and conventional rat chow containing 0.2% NaCl (Oriental Kobo, Tokyo) until the experiment, which was carried out after permission from Committee of Animal Experimentation, Hokkaido University School of Medicine. Rats were prepared as previously reported (Kawata et al., 1996). Briefly, rats were anesthetized with an intraperitoneal injection of thiobutabarbiturate sodium (Inactin, 100 mg/kg body wt; RBI, Natick, MA, USA) and placed on a feedback-controlled operating table that maintained body temperature constant at 37°C. After tracheotomy, a catheter was inserted from the right femoral artery into the abdominal aorta to monitor mean arterial pressure, using a pressure transducer (Nihonkoden, Tokyo, Japan) connected to an analog/digital (A/D) converter (MacLab, Melbourne, Australia) in a personal computer. Mean arterial pressure in the abdominal aorta was regarded as the renal perfusion pressure. The femoral vein was cannulated for administration of drug solution and for constant infusion of isotonic (0.9%) saline (1 ml/h per 100 g body wt). The left kidney was exposed through a flank incision, and embedded in a Lucite cup mounted on the operating table. The ureter was cannulated for free egress of urine. To control renal perfusion pressure level, an adjustable clamp was placed around the abdominal aorta above the origin of left renal artery. On completion of surgery, a 40–60 min equilibration period was allowed before initiating measurements. All the data were recorded and analysed on-line.

2.2. Drugs

All solutions were freshly prepared immediately before use in each experiment. Pinacidil (RBI) was dissolved at 0.05 mg/ml in 0.9% saline and administered by continuous i.v. infusion at the desired rate with a calibrated syringe pump (CFV-3200, Nihon-Koden, Tokyo, Japan). Glibenclamide (Wako, Osaka, Japan) was dissolved (10 mg/ml) in 0.9% saline and administered as an i.v. bolus injection (3 mg/kg body wt). This dose suppresses the hypotensive effect of K_{ATP} channel openers (Uchida et al., 1994).

2.3. Renal haemodynamics

To measure renal blood flow, the left renal artery was cleared and dissected from the renal vein and fitted with an electromagnetic flow probe connected to a flow meter (both Nihonkoden, Tokyo, Japan). Both mean arterial pressure and renal blood flow signals were passed to an A/D converter to obtain simultaneous on-line recordings throughout the experiments.

To obtain the dose of pinacidil giving maximum decrease in renal vascular resistance without severe hypotension, the dose/response relationship for the effect of pinacidil on mean arterial pressure, renal blood flow, and calculated renal vascular resistance was examined. Increasing the pinacidil dose from 0.03 to 0.06, 0.1, 0.15, or 0.3 mg/h per kg body wt decreased mean arterial pressure and renal vascular resistance significantly and dose dependently up to 0.15 mg h^{-1} kg^{-1} body wt in WKY and SHR (Fig. 1). This was the dose adopted as the experimental pinacidil dose for further studies.

To evaluate the renal haemodynamic action of pinacidil independently of any effect due to alteration of renal perfusion pressure, following the measurements under baseline conditions, renal haemodynamic parameters were again monitored with the renal perfusion pressure reduced by the aortic clamp by about 20% from baseline pressure. This is equivalent to the decline seen during the administration of pinacidil at 0.15 mg/h per kg body wt (Fig. 1). After the release of the aortic clamp, a 30-min equilibration period was allowed before beginning the pinacidil infusion. During drug infusion, infusion rate of saline was

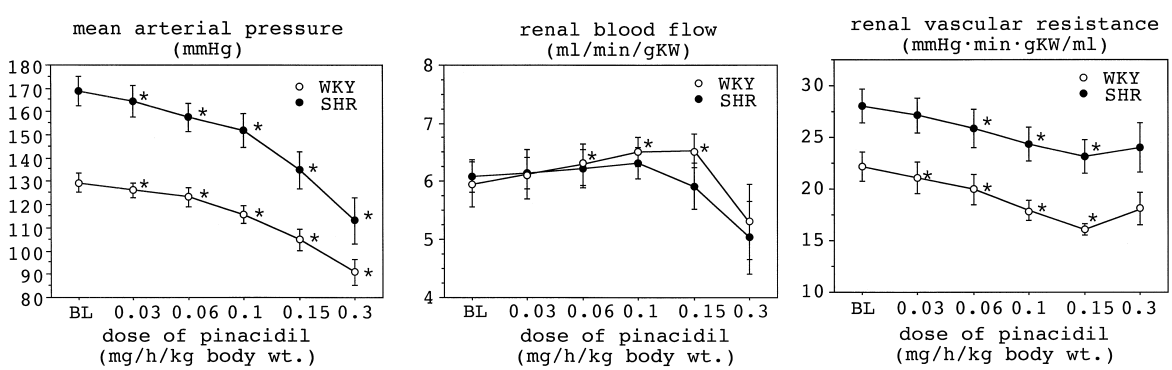

Fig. 1. Dose–response effect for the action of pinacidil on mean arterial pressure, renal blood flow and renal vascular resistance in Wistar Kyoto rats (WKY, -○-; $n = 7$) and spontaneously hypertensive rats (SHR, -●-; $n = 7$).

adjusted to give the total infusion rate as 1 ml/h per 100 g body wt.

2.4. Micropuncture studies

All micropuncture procedures and measurements were started after stabilization of blood pressure. Surgical, micropuncture and microperfusion procedures were as reported elsewhere (Kawata et al., 1996). Briefly, the left kidney was fixed in a Lucite cup with 2% agar and the kidney surface immersed with warmed (37°C) saline. Proximal tubular segments were identified, and the middle proximal segments were completely blocked by solid wax (Merck, Darmstadt, Germany). The most proximal segment upstream from the wax block was gently punctured with a micropipette (o.d. 2 μm) filled with 2 M NaCl solution stained with lissamine green (Chroma, Köngen, Germany) and mounted in a servo–null micropressure system (WPI, Boca Raton, FL, USA) to monitor stop-flow pressure (P_{sf}). After the stabilization of P_{sf}, a perfusion pipette (o.d. 10 μm) filled with artificial tubular fluid (Na^+, 140 mM; Cl^-, 140 mM; K^+, 4 mM; Ca^{2+}, 4 mM; HCO_3^-, 8 mM; Urea, 7.5 mM) and mounted in a microperfusion pump (Effenberger, Attel, Germany) was inserted into a late proximal segment. The alteration of P_{sf} in response to perfusion of Henle's loop at various rates (0–40 nl/min) was regarded as the tubuloglomerular feedback response of the nephron. Measurements from any nephron showing leakage of the stained solution was discarded.

2.5. Calculations

Renal vascular resistance was calculated as quotient of renal perfusion pressure and renal blood flow. The characteristic tubuloglomerular feedback response was assessed quantitatively using the analytical method involving least-square curve fitting (Schnermann and Briggs, 1989). Loop of Henle perfusion rate (V_{lp}) and P_{sf} are related in the so-called logistic equation:

$$P_{sf} = P_{sf,0} + \frac{\Delta P_{sf,max}}{1 + e^{k(V_{1/2} - V_{lp})}}$$

by four additional parameters: $P_{sf,0}$, P_{sf} in the absence of loop perfusion; k, an exponential constant describing the width of the perfusion interval over which the P_{sf} responds; $\Delta P_{sf,max}$, the maximum decrease in P_{sf}; and $V_{1/2}$, the value of V_{lp} at which the response is half-maximal. The P_{sf} data for five loop perfusion rates for each nephron were fitted using a nonlinear least-square procedure.

2.6. Statistical analyses

All values are means ± S.E. Student's non paired *t*-test was used to test for statistical significance of differences of the values between WKY or SHR. Student's paired *t*-test was used to assess renal haemodynamic data obtained during the maneuvers within a rat group. Analysis of variance (ANOVA) was used to test for statistical significance of differences among the micropuncture data observed during each treatment within a rat group. $P < 0.05$ was regarded as significant.

3. Results

3.1. Renal haemodynamics

The effects of pinacidil and/or glibenclamide on renal haemodynamics in WKY and SHR are shown in Tables 1

Table 1
Effect of pinacidil on renal hemodynamic parameters (RPP, mean renal perfusion pressure; RBF, renal blood flow measured by electromagnetic flow meters; RVR, renal vascular resistance calculated from RPP and RBF) in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR)

	n	RPP (mmHg)	%ΔRPP (%)	RBF (ml/min per g kw)	%ΔRBF (%)	RVR (mmHg min/ml)	%ΔRVR (%)
WKY							
BL	8	122 ± 4		6.40 ± 0.29		19.1 ± 0.7	
CL		107 ± 1[a]	−11.4 ± 2.9	5.24 ± 0.36[a]	−18.1 ± 5.0	21.2 ± 1.8	11.1 ± 8.3
PN		106 ± 4[a]	−12.6 ± 2.9	6.79 ± 0.21[b]	6.88 ± 4.06[b]	15.7 ± 0.6[a,b]	−17.9 ± 2.3[b]
SHR							
BL	5	158 ± 6[c]		6.30 ± 0.31		25.1 ± 1.0[c]	
CL		134 ± 5[a,c]	−14.9 ± 2.2	5.19 ± 0.27[a]	−17.0 ± 5.4	26.1 ± 1.7[c]	3.85 ± 5.76
PN		137 ± 7[a,c]	−13.2 ± 1.4	5.98 ± 0.32[a,b]	−4.94 ± 3.78[b]	23.1 ± 1.5[b,c]	−8.13 ± 3.98[b,c]

Measurements were conduced in baseline condition (BL), during aortic clamp (CL), and during pinacidil infusion (PN, 1.5 mg/kg body wt).
Abdominal aorta was clamped to give equivalent level of RPP to that during pinacidil infusion.
The percentage changes from each baseline value are shown as %ΔRPP, %ΔRBF and %ΔRVR.
Values are means ± S.E.
[a]Significant difference by paired *t*-test from corresponding value in baseline condition.
[b]Significant difference by paired *t*-test from corresponding value in aortic clamp.
[c]Significant difference by non-paired *t*-test from corresponding value in WKY.

Table 2
Effect of glibenclamide on renal hemodynamic parameters (RPP, mean renal perfusion pressure; RBF, renal blood flow measured by electromagnetic flow meters; RVR, renal vascular resistance calculated from RPP and RBF) in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR)

	n	RPP (mmHg)	%ΔRPP (%)	RBF (ml/min per g kw)	%ΔRBF (%)	RVR (mmHg min/ml)	%ΔRVR (%)
WKY							
BL	6	127 ± 4		6.28 ± 0.19		20.4 ± 1.1	
GC		126 ± 4	−0.81 ± 0.52	6.45 ± 0.18	2.67 ± 0.76	19.7 ± 1.1	−3.36 ± 0.97
GC/PN		119 ± 2[a,b]	−6.38 ± 1.7[b]	5.68 ± 0.10[a,b]	−9.39 ± 1.90[b]	21.0 ± 0.7	−3.58 ± 3.1
SHR							
BL	6	160 ± 3[c]		6.02 ± 0.38		27.2 ± 1.9[c]	
GC		161 ± 3[c]	0.8 ± 0.6	6.19 ± 0.38	2.95 ± 0.91	26.6 ± 1.7[c]	−2.08 ± 0.81
GC/PN		148 ± 3[a,b,c]	−7.8 ± 1.3[b]	5.84 ± 0.20	−1.97 ± 3.46	25.4 ± 0.9[c]	−5.31 ± 3.91

Measurements were conduced in baseline condition (BL), after bolus injection of glibenclamide (GC, 3 mg/kg body wt), and during subsequent pinacidil infusion (0.15 mg/kg body wt), GC/PN.
The percentage changes from each baseline value are shown as %ΔRPP, %ΔRBF and %ΔRVR.
Values are means ± S.E.
[a]Significant difference by paired *t*-test from corresponding value in baseline condition.
[b]Significant difference by paired *t*-test from corresponding value in glibenclamide infusion.
[c]Significant difference by non-paired *t*-test from corresponding value in WKY.

and 2. In both rat groups, renal perfusion pressure was kept at levels seen during infusion of pinacidil by the aortic clamp, in both cases significantly lower than in baseline. Pinacidil reduced renal vascular resistance significantly in both rat groups and the vasodilative effect is significantly greater in WKY than SHR (Table 1).

Bolus injection of glibenclamide did not alter renal perfusion pressure or renal haemodynamics in either rat group, and subsequent pinacidil administration reduced renal perfusion pressure but not renal vascular resistance (Table 2). The increase in renal blood flow and reduction in renal vascular resistance observed in WKY treated with pinacidil alone (Table 1) were attenuated by pretreatment with glibenclamide (Table 2).

3.2. Micropuncture study

The effects of pinacidil and/or glibenclamide on P_{sf} and the tubuloglomerular feedback response are summarized in Tables 3 and 4. Alterations of renal perfusion pressure were almost equivalent to those observed in renal haemodynamic study (Tables 1 and 2). During pinacidil

Table 3
Effect of pinacidil on renal micropuncture data in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR)

	n (tubule/rat)	RPP (mmHg)	$P_{sf,0}$ (mmHg)	$P_{sf,40}$ (mmHg)	ΔP_{sf} (mmHg)	$\%\Delta P_{sf}$ (%)	$f'(V_{1/2})$	$V_{1/2}$ (nl/min)
WKY								
BL	21/6	114 ± 2	38.7 ± 2.1	30.4 ± 2.0	−8.30 ± 0.59	22.2 ± 1.9	−0.24 ± 0.05	18.1 ± 2.4
CL	15/6	99 ± 2[a]	33.7 ± 2.1	27.0 ± 2.0	−6.62 ± 0.64	20.3 ± 2.1	−0.23 ± 0.05	17.7 ± 3.6
PN	16/6	100 ± 4[a]	42.5 ± 2.1[c]	33.5 ± 1.9[c]	−9.08 ± 0.95	21.5 ± 1.9	−0.26 ± 0.07	20.5 ± 3.0
SHR								
BL	22/7	161 ± 5[d]	41.0 ± 1.7	30.6 ± 1.3	−10.76 ± 0.60[d]	26.0 ± 1.1[d]	−0.51 ± 0.05[d]	20.7 ± 1.7
CL	14/7	137 ± 5[a,d]	35.1 ± 1.6[b]	27.9 ± 1.0	−5.83 ± 0.68[b]	17.0 ± 1.5[b]	−0.27 ± 0.06[b]	15.6 ± 1.5
PN	18/7	137 ± 8[a,d]	32.7 ± 0.6[b,c]	25.7 ± 0.5[b,d]	−6.92 ± 0.29[b,d]	21.1 ± 0.7[b]	−0.30 ± 0.04[b]	19.7 ± 2.5

The middle proximal segment were blocked by solid wax and pressure in the tubular segment upstream from the wax block was monitored (P_{sf}, stop flow pressure) during microperfusion of Henle's loop at various rates (0–40 nl/min) with artificial tubular fluid.
The changes from P_{sf} at zero loop flow ($P_{sf,0}$) to P_{sf} at 40 nl/min ($P_{sf,40}$) are shown as ΔP_{sf} in absolute value and $\%\Delta P_{sf}$ in percent value.
The loop flow rate ($V_{1/2}$) and the slope ($f'(V_{1/2})$) at which the tubuloglomerular feedback response is half-maximal were calculated using least-squares non-linear regression analysis of P_{sf} data.
Measurements were conduced in baseline condition (BL), during aortic clamp (CL), and during pinacidil infusion (PN, 0.15 mg/kg body wt).
Values are means ± S.E.
[a]Significant difference by paired *t*-test from corresponding value in baseline condition.
[b]Significant difference by ANOVA from corresponding value in baseline condition.
[c]Significant difference by ANOVA from corresponding value in aortic clamp.
[d]Significant difference by non-paired *t*-test from corresponding value in WKY.

Table 4
Effect of glibenclamide on renal micropuncture data in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR)

	n (tubule/rat)	RPP (mmHg)	$P_{sf,0}$ (mmHg)	$P_{sf,40}$ (mmHg)	ΔP_{sf} (mmhg)	$\%\Delta P_{sf}$ (%)
WKR						
BL	11/5	116 ± 3	40.6 ± 1.1	31.8 ± 1.3	−8.42 ± 0.73	21.0 ± 1.8
GC	8/5	117 ± 3	38.8 ± 1.7	30.1 ± 2.0	−8.65 ± 1.19	22.5 ± 3.2
GC/PN	10/5	110 ± 2[a]	38.2 ± 0.9	30.2 ± 1.2	−7.95 ± 0.53	21.0 ± 1.7
SHR						
BL	10/6	150 ± 2[d]	40.6 ± 1.0	29.4 ± 0.9	−11.4 ± 0.8[d]	28.1 ± 1.8[d]
GC	12/6	151 ± 3[d]	38.4 ± 1.3	27.7 ± 1.3	−10.8 ± 0.4[d]	28.4 ± 1.4[d]
GC/PN	8/6	141 ± 3[a,d]	35.2 ± 1.8[b]	27.1 ± 1.4	−8.07 ± 0.81[b,c]	23.1 ± 2.5[b]

The middle proximal segment were blocked by solid wax and pressure in the tubular segment upstream from the wax block was monitored (P_{sf}, stop-flow pressure) during microperfusion of Henle's loop at various rates (0–40 nl/min) with artificial tubular fluid.
The changes from P_{sf} at zero loop flow ($P_{sf,0}$) to P_{sf} at 40 nl/min ($P_{sf,40}$) are shown as ΔP_{sf} in absolute value and $\%\Delta P_{sf}$ in percent value.
Measurements were conduced in baseline condition (BL), after bolus injection of glibenclamide (GC, 3 mg/kg body wt), and during subsequent pinacidil infusion (0.15 mg/kg body wt), GC/PN.
Values are means ± S.E.
[a]Significant difference by paired t-test from corresponding value in baseline condition.
[b]Significant difference by ANOVA from corresponding value in baseline.
[c]Significant difference by ANOVA from corresponding value in glibenclamide infusion.
[d]Significant difference by non-paired t-test from corresponding value in WKY.

infusion, $P_{sf,0}$ changed almost in parallel with renal perfusion pressure in SHR, but not in WKY. In the latter $P_{sf,0}$ was significantly elevated, while renal perfusion pressure decreased to a level comparable to that during aortic clamp. The change of P_{sf} with loop perfusion is depicted in Fig. 2. The tubuloglomerular feedback response in ΔP_{sf} or in $\%\Delta P_{sf}$ was not altered during aortic clamp or with pinacidil in WKY but were significantly attenuated in SHR, for which the corresponding changes can be seen in the slope of the tubuloglomerular feedback response curve at operating point ($f'(V_{1/2})$) (Table 3). The curve constructed from the values shown in Fig. 2 is depicted in Fig. 3, where the operating point, i.e., that value of P_{sf} at a loop perfusion rate of $V_{1/2}$, which corresponds to the steady-state glomerular capillary pressure (Schnermann and Briggs, 1989), revealed no significant change during aortic clamp or pinacidil treatment in WKY. However, in SHR, operating point was significantly reduced during both aortic clamp and pinacidil treatments, where the changes in renal perfusion pressure were almost equivalent (Table 3).

Pretreatment with glibenclamide had no significant effect in $P_{sf,0}$ or tubuloglomerular feedback responses in either rat group (Table 4). In WKY, subsequent infusion of pinacidil moderately decreased renal perfusion pressure, but $P_{sf,0}$ and tubuloglomerular feedback responses were not different from those during baseline or glibenclamide

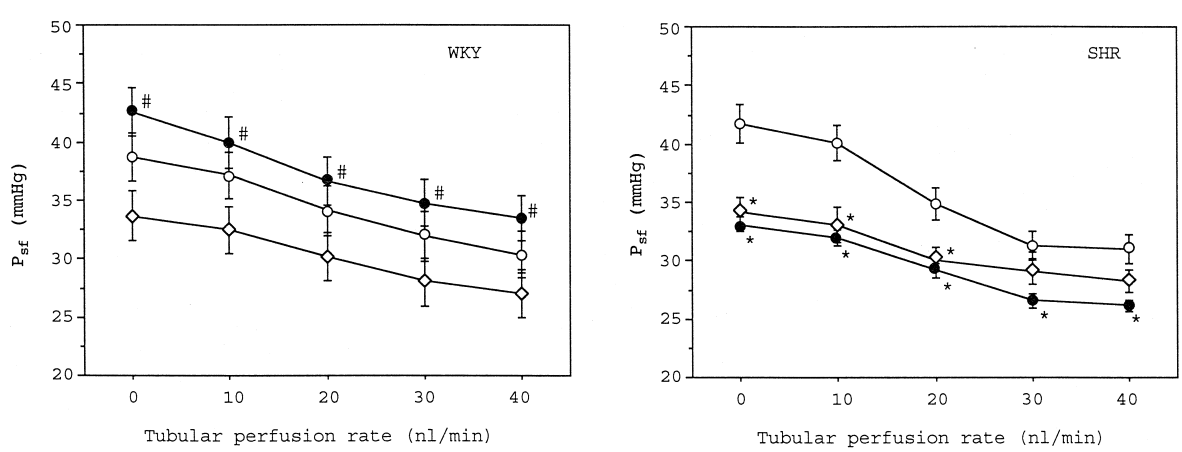

Fig. 2. Effect of pinacidil infusion (0.15 mg h^{-1} kg^{-1} body wt) on tubuloglomerular feedback response of proximal stop-flow pressure (P_{sf}) in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). P_{sf} during tubular perfusion with artificial tubular fluid is shown under baseline conditions (-○-; $n = 16$), during aortic clamp (-◇-; $n = 13$), and during pinacidil infusion (-●-; $n = 15$). Symbols are means ± S.E. $^{\star}P < 0.05$ vs. baseline; $\#P < 0.05$ vs. clamp in each series.

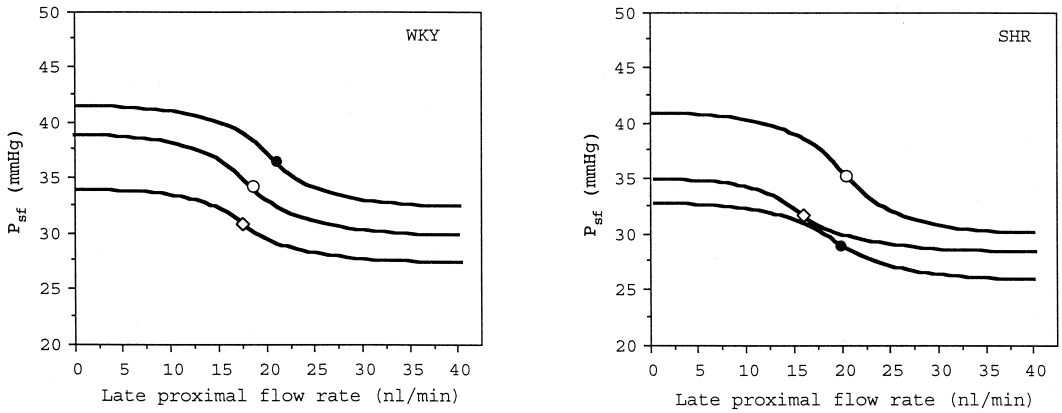

Fig. 3. Relationship between P_{sf} and rate of tubular perfusion with artificial tubular fluid in Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Curves are obtained from least-squares fitting of data from Fig. 2. Symbols indicate computed P_{sf} at half-maximal response in baseline (-○-), during clamp (-◇-), and during infusion of pinacidil (-●-).

(Table 4). In contrast, in SHR, these parameters were significantly decreased compared with baseline or glibenclamide (Table 4).

4. Discussion

4.1. Renal effect of K_{ATP} channel opener

Opening of K_{ATP} channels hyperpolarizes vascular smooth muscle, leading to inhibition of L-type Ca^{2+} channels and vasodilatation (Nelson and Quayle, 1995). Although the renal vasculature is also sensitive to the in vivo application of K_{ATP} channel openers (Morimoto et al., 1987), the magnitude of vasodilatation in kidney is less than that in coronary mesenteric, or femoral arteries (Ogawa et al., 1993). Furthermore, the systemic effects in vivo application of K_{ATP} channel openers may affect renal perfusion pressure (Fig. 1) to an extent that would conceal any specific renal haemodynamic effects of the channel opener per se. We thus compared the renal or glomerular haemodynamic parameters at equivalent renal perfusion pressure and observed a significant decrease in renal vascular resistance and increase in renal blood flow (Table 1). This renal vasodilation was attenuated significantly by prior treatment with glibenclamide, suggesting that the major part of the pinacidil's haemodynamic effects can be attributed to the opening of the native K_{ATP} channels.

Direct evidence of afferent arteriolar dilatation by K_{ATP} channel openers has been obtained in the hydronephrotic kidney (Fleming et al., 1987; Reslerova and Loutzenhiser, 1995). Other studies have demonstrated the presence of the K_{ATP} channels in the glomerular afferent arteriole (Lorenz et al., 1992; Metzger and Quast, 1996). Indeed, the significant increase in P_{sf} in WKY (Table 3 and Fig. 2) strongly suggests vasodilatation of the preglomerular vasculature by activation of K_{ATP} channels. However, a recent study in the hydronephrotic rat kidney has shown that pinacidil can also dilate efferent arteriole (Reslerova and Loutzenhiser, 1995), presumably by different mechanisms not dependent on L-type Ca^{2+} channel activity. Thus, the mechanism of the effect of K_{ATP} channel openers on the renal vasculature may not be straight forward and the renal haemodynamic alterations seen in the present study may reflect, in part, effects on the efferent arteriole.

4.2. Why is the tubuloglomerular feedback mechanism unaffected?

This is the first study evaluating tubuloglomerular feedback, a unique system regulating renal haemodynamics, in the presence of a K_{ATP} channel opener. Pinacidil at a dose that significantly decreased renal vascular resistance had no significant effect on the tubuloglomerular feedback mechanism in either SHR or WKY. The effect of inhibition of K_{ATP} channels from the tubular lumen on the activity of tubuloglomerular feedback has been recently investigated (Vallon et al., 1997). In that study, the response was attenuated when the macula densa segment was perfused with artificial tubular fluid containing a very high concentration (100 μM) of the K_{ATP} channel inhibitor, U37883A (4-morpholinecarboximidine-*N*-1-adamantyl-*N′*-cyclohexylhydrochloride). The authors suggest that the lowered K^+ concentration of the tubular perfusate due to decreased K^+ influx through the inhibition of tubular K_{ATP} channels would limit the activity of Na^+–$2Cl^-$–K^+ cotransporter in the macula densa, thus interfering with the sensing step of the tubuloglomerular feedback mechanism. Although we did not evaluate K^+ concentration in the tubular perfusate at the macula densa, tubuloglomerular feedback activity was not affected by i.v. glibenclamide or pinacidil in either group of rats (Tables 3 and 4). Since, however, tubular K_{ATP} channels have higher

IC_{50} (250 μM) for glibenclamide and higher EC_{50} (100 μM) for K_{ATP} channel openers than vascular K_{ATP} channels (IC_{50}; 0.025 μM and $EC_{50} < 0.5$ μM, respectively) (Quast, 1996), the drugs at the doses we used may have only limited effects on the composition of the tubular perfusate at the macula densa, especially at high perfusion rate.

In the tubuloglomerular feedback mechanism, components of the signal transmission from the sensing site, the macula densa, to the effector site, the afferent arteriole in the vicinity of the glomerulus, are thought to be dependent on the cell depolarization process, in which the L-type Ca^{2+} channel plays an important role (Kawata et al., 1997). Modulation of L-type Ca^{2+} channel activity by K_{ATP} channel openers in the renal vasculature or juxtaglomerular apparatus thus would be expected to have a substantial effect on the tubuloglomerular feedback mechanism. In the present study, however, pinacidil had no significant effect on tubuloglomerular feedback activity (Table 3 and Fig. 2), while renal vascular resistance was significantly reduced. Since K_{ATP} channel openers are known to cause afferent arteriolar vasodilatation (Fleming et al., 1987; Reslerova and Loutzenhiser, 1995), a possible explanation is that affinity of the agent to K_{ATP} channels or density of these channels may be lower at the site of tubuloglomerular feedback operation than in other preglomerular vascular segments. Differences in K_{ATP} channel activity between vascular beds have been shown in other studies (McCarron et al., 1991; McPherson and Stork, 1992). A further possible explanation is the increase in plasma renin activity caused by K_{ATP} channel openers (Richer et al., 1990; Pratz et al., 1991). This would increase angiotensin II production and activate tubuloglomerular feedback (Schnermann and Briggs, 1986) offsetting the attenuation brought about by inhibition of L-type Ca^{2+} channel activity by the opening of K_{ATP} channels in the juxtaglomerular apparatus (Metzger and Quast, 1996). Our experiments do not address these questions, and hence the explanations remain speculative.

4.3. The possible diversity of the K_{ATP} channel action in normotension and hypertension

The second novel finding of this study is that the effect of pinacidil on renal vascular resistance or P_{sf} is attenuated in SHR. In hypertension, the activity of L-type Ca^{2+} channels (Rusch and Hermsmeyer, 1988; Ohya et al., 1993; Wilde et al., 1994) and Ca^{2+}-dependent K^+ channels (Rusch et al., 1992; Rusch and Runnells, 1994) is increased compared with normotensive controls. The elevated vascular tone in hypertension is attributed to such alterations in channel activity. With regard to K_{ATP} channel activity in hypertension the literature is not consistent. Thus the dilator responses to the K_{ATP} channel opener are similar in the tail artery of stroke prone SHR (SHR-SP) and WKY (Furspan and Webb, 1993), but impaired in the cranial artery of SHR-SP (Kitazono et al., 1993) and in the mesenteric artery of SHR (Ohya et al., 1996) compared with WKY. The attenuated effects of pinacidil on renal haemodynamics in SHR observed in the present study are consistent with the latter studies.

Treatment with glibenclamide did not affect renal haemodynamics per se, but attenuated the effects of pinacidil (Table 2). Unlike coronary or mesenteric vessels, renal arteriolar diameter was not affected by glibenclamide (Loutzenhiser and Parker, 1994; Reslerova and Loutzenhiser, 1995). Therefore, K_{ATP} channels might make only a marginal contribution to the basal tone of the renal vasculature under normal conditions, such as in WKY in our study. Although K_{ATP} channels have a low steady-state conductance and are activated in metabolic failure, such as during hypoxia (Loutzenhiser and Parker, 1994), channel activity may also be modulated by endogenous autacoids and may contribute to the maintenance of resting potential whereby the extent of this contribution may differ between organs and different systemic conditions (Olsson and Pearson, 1990; Nelson and Quayle, 1995; Clapp and Tinker, 1998). Since, the attenuated K_{ATP} channel activity in SHR can be reversed by long-term anti-hypertensive treatment (Ohya et al., 1996), the alteration of channel function in the renal vasculature of SHR, as suggested in our study, may also be the consequence of hypertension per se or the altered status of the endogenous modulators of the channels (adenosine, calcitonin gene related peptide, and epinephrine) (Nelson and Quayle, 1995) in hypertension. Furthermore, such alteration in channel activity may contribute, at least in part, to the elevated renal vascular tone, especially in the preglomerular segment, in hypertension.

4.4. Summary

This study demonstrates a significant contribution of K_{ATP} channels to renal vascular function in vivo, the magnitude of which may differ in renal vascular segments and between normotension and hypertension. The tone of the preglomerular vessels is regulated by intrinsic myogenic mechanism and the tubuloglomerular feedback mechanism (Navar, 1998). Activation of K_{ATP} channels significantly interferes with the former mechanism (Loutzenhiser and Parker, 1994), which can regulate the most part of the renal vascular resistance. Thus the attenuated action of K_{ATP} channels may greatly interfere with the vasodilatory response to autacoids and lead to the elevated renal vascular resistance in hypertension. The molecular nature of the vascular K_{ATP} channels has not yet been established, but smooth muscle specific isoforms have been suggested (Clapp and Tinker, 1998). The exact nature of the alteration of vascular K_{ATP} channels and its physiological and pathophysiological relevance in the renal vascular function must be established further.

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