Original Article

Visualisation of the Effects of Dilazep on Rat Afferent and Efferent Arterioles *In Vivo*

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Although the effects of dilazep hydrochloride (dilazep), a nucleoside transport inhibitor, have been examined, there have been no visualisation studies on the physiological effects of dilazep on the glomerular arterioles. The purpose of this study was to visualise and evaluate the effects of dilazep and consequently the effects of adenosine, which dilazep augments by measuring glomelurar diameters, renal blood flow and resistance in rats *in vivo*. We time-sequentially examined afferent and efferent arteriolar diameter changes using an intravital videomicroscope and renal blood flow. We administered dilazep at a dose of 300 μg/kg intravenously. To further investigate the effects of dilazep, rats were pre-treated with 8-*p*-sulfophenyl theophylline (a nonselective adenosine receptor antagonist), 8-cyclopentyl-1,3-dipropylxanthine (an A1 receptor antagonist), or 3,7-dimethyl-1-propargylxanthine (an A2 receptor antagonist). Dilazep constricted the afferent and efferent arterioles at the early phase and dilated them at the later phase, with the same degree of vasoconstrictive and vasodilatory effect on both arterioles. A1 blockade abolished vasoconstriction and augmented vasodilatation at the later phase and A2 blockade abolished vasodilatation and augmented vasoconstriction at the early phase. Non-selective blockade abolished both early vasoconstriction and later vasodilatation. In conclusion, adenosine augmented by dilazep constricted the afferent and efferent arterioles of the cortical nephrons at the early phase and dilated both arterioles at the later phase *via* A1 and A2 adenosine receptor activation, respectively. That the ratio of afferent to efferent arteriolar diameter was fairly constant suggests that intraglomerular pressure is maintained in the acute phase by adenosine despite the biphasic flow change. (*Hypertens Res* 2008; 31: 315–324)

***Key Words*: adenosine, dilazep, kidney, vasoconstriction, visualisation**

Introduction

Adenosine, which is generated locally in tissue by conditions that produce hypoxia, ischaemia, or inflammation, mediates a variety of physiological functions. Traditionally, adenosine has been thought to play a critical role in the local regulation of blood flow (*1*). In most blood vessels, adenosine elicits marked vasodilatation. Vasoconstrictive A1 receptors are, however, present in blood vessels of the kidney in addition to vasodilatory A2 receptors, and this has made the renal vascular actions of adenosine comparatively complex (*2*). A2 receptors are subdivided into A2a and A2b receptors. A2a receptors induce relaxation of vascular smooth muscle cells and inhibition of neutrophil function. The former action, relaxation of smooth muscle cells plays a role in renal physiology. While A2b receptors are involved in inhibition of mesangial and vascular smooth muscle cell growth, both actions contribute to renal physiology but they have nothing to do with the present study which focuses on acute effect of dilazep resulting in glomerular arteriolar diameter changes. It has been reported that A1 receptors are predominantly expressed in the afferent arterioles. It has been suggested that A2 receptors are present in both afferent and efferent arterioles, and that they mediate important haemodynamic effects (*1*). A2 receptors, mostly A2b receptors, are present in all preglomerular vessels and in the descending vasa recta. For the sake of simplicity, we will refer to A2a receptors as A2 recep-

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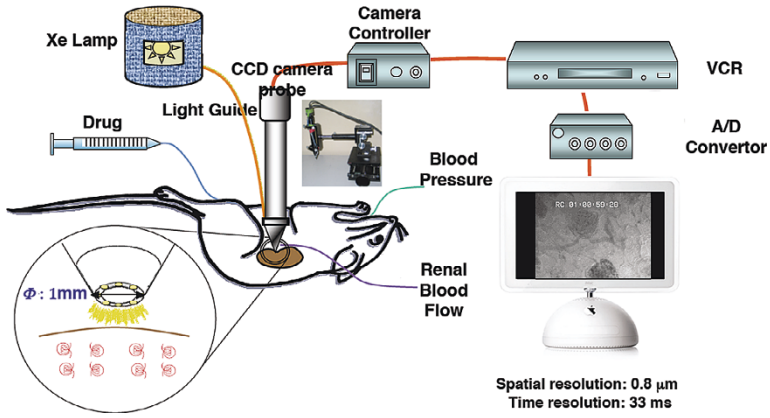

Fig. 1. *Experimental set-up. The intravital videomicroscope system consists of a CCD camera probe, a light source, a camera controller, a videocassette recorder, an AD converter and an Apple computer. The spatial resolution of this system for a static image is 0.8 μm and the spatial resolution for a moving object is 0.4 μm. Its time resolution is 33 ms. From the incision on the surface of the left kidney, the probe tip is introduced into the cortex by an xyz stage to visualise the renal microcirculation.*

Table 1. Body Weights and Haemodynamic Data before Dilazep Hydrochloride Administration

	Saline	300 mg/kg dilazep	A1 and A2 blocakde 8-sPT	A1 blockade DPCPX	A2 blockade DMPX
No. of rats	11	11	7	8	7
No. of glomeruli	22	23	16	24	12
Body weight (g)	344±17	359±20	354±12	353±12	355±7
Left renal arterial flow (mL/min)	2.7±0.1	2.7±0.2	2.7±0.3	2.6±0.1	2.7±0.3
Mean arterial pressure (mmHg)	97±2	96±2	95±3	99±3	98±3

Values are expressed as means±SEM. 8-sPT, 8-*p*-sulfophenyl theophylline; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMPX, 3,7-dimethyl-1-propargylxanthine.

tors in this study, unless otherwise specified. In the coronary vasculature, adenosine is a main vasodilatory mediator for arterioles with a diameter of less than approximately 50 μm. Adenosine preferentially targets the arterioles. The mean size of the glomerular vessels in the present study was less than 20 μm, where dilatation is considered to be caused by adenosine. Dilazep (COMELIAN®; Kowa Co. Ltd., Nagoya, Japan), a nucleoside transport inhibitor (*3*) and an anti-platelet agent, was first developed for the treatment of angina pectoris. There have been several reports that have dealt with the effects of dilazep on the renal microcirculation, but none has measured glomerular arteriolar diameters. Nagase *et al*. reported that dilazep has an anti-albuminuric effect (*4*). Yukimura *et al*. examined dilazep-induced changes in renal flow in dogs and showed that the renal vascular effects of dilazep may be exerted by augmentation of endogenous adenosine and mediated through adenosine receptors (*5*). Kawabata *et al*. have also reported in their clearance and micropuncture study that extracellular adenosine augmented by dilazep dilated both afferent and efferent arterioles, probably *via* A2 receptors (*6*). As the coronary vasodilator dilazep augments endogenous adenosine actions, it may aid in elucidating the physiological roles of adenosine in renal haemodynamics.

The renal microcirculation has been examined in several visualisation studies that have measured the microvascular diameters in juxtamedullary nephrons (*7–9*), hamster cheek pouch allografts of renal tissue (*10*), and the hydronehrotic kidney model (*11–13*). These studies were based on either isolated preparation or pathological animal models, and thus their results may differ from those of a study under normal physiological conditions. Glomerular arteriolar diameter changes have not been confirmed yet under physiological conditions due to the difficulty of gaining access to the renal microcirculation. Recently, we have developed a CCD intravital videomicroscope to overcome such technical difficulties in observing the renal microcirculation (*14*, *15*).

We hypothesize that, despite the difference in the distribution of A1 and A2 receptors, the changes in vascular diameter in response to dilazep are associated with the maintenance of a constant glomerular pressure. This study was designed to visualise and test this hypothesis by measuring afferent and efferent diameters in rats under physiological conditions using our intravital videomicroscope. In addition, three adenosine antagonists—A1 inhibitor, A2 inhibitor and non-

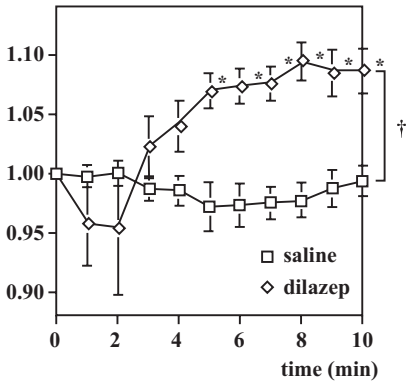

Fig. 2. *Effects of dilazep on renal arterial flow. Flow changes are expressed as a ratio. Biphasic renal arterial flow change with early decrease and late increase was observed by 300 μg/kg dilazep administration. Open squares, saline; open diamonds, 300 μg/kg dilazep; †p < 0.05 vs. saline (ANOVA); *p < 0.05 vs. basal (t-test).*

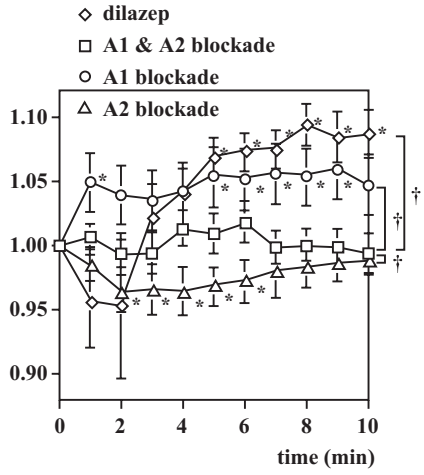

Fig. 3. *Effects of A1 and/or A2 receptor on renal arterial flow. Flow changes are expressed as a ratio. A1 receptor blockade abolished the early decrease in flow. A2 receptor blockade abolished the late flow increase. A1 and A2 receptor blockade abolished both the flow decrease and increase. Open diamond, 300 μg/kg of dilazep; open square, A1 and A2 receptor blockade; open circle, A1 receptor blockade by DPCPX (1 mg/kg); open triangle, A2 receptor blockade by DMPX (1 mg/kg); †p < 0.05 vs. A1 and A2 receptor blockade by 8-sPT (10 mg/kg) (ANOVA), *p < 0.05 vs. basal (t-test).*

selective adenosine inhibitor—were administered to examine the mechanism of action of dilazep.

Methods

Pencil-Type CCD Intravital Videomicroscope

Details of the pencil-type CCD intravital videomicroscope have been described previously (*14*, *16*). Briefly, the videomicroscope system has a sharp pencil–like probe, which contains a CCD image sensor of 0.5 by 0.5 square inches with 640 × 480 pixels. The system (Fig. 1) consists of a pencil lens probe (ø: 1 mm) with eight annularly arranged light guides, a CCD camera (Nihon Kohden, Tokyo, Japan), a light source, a videocassette recorder and a computer for image analysis (Power Macintosh G4; Apple Computer, Cupertino, USA). The spatial resolution of this system for a static image is 0.8 μm and the spatial resolution for a moving object is 0.4 μm for 520 magnification. Its depth of focus is about 50 μm and its time resolution is 33 ms, 30 frames/s.

Animal Preparation

Forty-four male Wistar rats of 12 to 16 weeks of age were first anaesthetized by ether inhalation and then injected with thiobutabarbital (100 mg/kg) and placed on an electric blanket with a feedback regulator to maintain the body temperature at 37°C. The right femoral vein was cannulated for drug administration, and the right carotid artery was cannulated for blood pressure monitoring. The left kidney was exposed *via* lateral incision. A transonic blood flowmeter (model T206; Transonic Systems, Ithaca, USA) was set around the left renal artery (Fig. 1). The experimental protocols were approved by the Committee on Animal Research of Kawasaki Medical School, and the care of all animals used in these experiments complied with the guidelines of the National Institutes of Health. The experimental procedures were in accordance with the guidelines of our institution and those of the National Research Council.

Measurement of Vascular Diameters

The tip of the probe was introduced into the superficial layer of the cortex through an incision on the kidney surface. After a clear image of a glomerulus was obtained, the probe was withdrawn for several tens of micrometers. The image was refocused so that the probe did not compress the glomerular afferent and efferent arterioles. Obtained images were recorded and analysed off-line afterwards by NIH image processing software. Afferent and efferent arterioles were differentiated by the direction of movement of blood cells and/or of plasma pockets to and from a glomerulus visually. The time-sequential images of the renal microcirculation were analysed by the computer included inthe videomicroscope system. The vascular images of the afferent and efferent arterioles were analysed in a freeze-frame modality. The internal diameters were determined either by manual border tracing or by automated edge detection using a density profile, averaging at least three measurements at every minute from the beginning of drug administration.

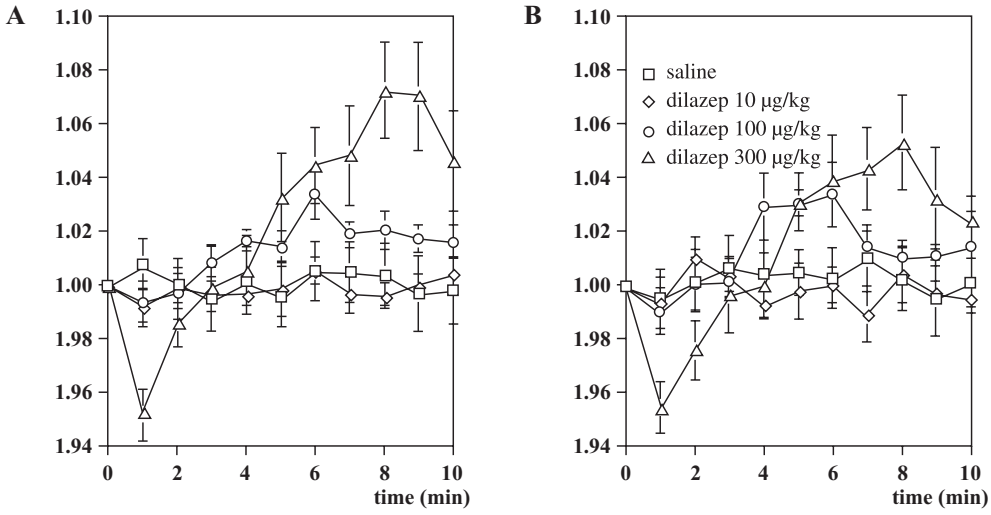

Fig. 4. *Effects of dilazep on afferent and efferent arterioles. The changes in diameter are expressed as ratios. A: Afferent arterioles: biphasic afferent arteriolar diameter change with early vasoconstriction and late vasodilatation was observed in a dose-dependent manner. It was greatest following intravenous administration of 300 μg/kg dilazep (n = 23) and both early vasoconstriction and late vasodilatation were significant. By 100 μg/kg dilazep administration, such biphasic diameter change was similar but less pronounced (number of rats = 9). There was no significant difference in the afferent diameter among the administration of saline, 10 μg/kg dilazep (number of rats = 9) and 8-sPT (number of rats = 16). B: Efferent arterioles: biphasic efferent arteriolar diameter change was observed in a dose-dependent manner. It was greatest following intravenous administration of 300 μg/kg dilazep. After 100 μg/kg dilazep administration, such biphasic diameter change was similar but less pronounced. There was not any significant efferent diameter change for saline and 10 μg/kg dilazep (number of rats = 9) compared with that of 8-sPT (number of rats = 16) administration. Open square, saline; open diamond, 10 μg/kg of dilazep; open circle, 100 μg/kg; open triangle, 300 μg/kg.*

Experimental Protocol

After an approximately 30-min stabilisation period, dilazep was administered at a dose of 300 μg/kg in 0.1 mL saline *via* the right femoral vein. We also examined administering 10 and 100 μg/kg of dilazep before determining that 300 μg/kg was the appropriate dose. We previously confirmed that this dose does not affect systemic blood pressure (*5*). Administration of 500 μg/kg of dilazep decreased main arterial blood pressure in the preliminary studies, and thus this dose was not used in the present study. An equivalent volume of saline was administered as a control. To investigate the effects of dilazep further, three adenosine receptor antagonists were administered as pre-treatments: 8-*p*-sulfophenyl theophylline (8-sPT, 10 mg/kg), a nonselective adenosine receptor antagonist, was administered intravenously; 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 mg/kg), a selective A1 receptor antagonist, was administered intraperitoneally; and 3,7-dimethyl-1-propargylxanthine (DMPX, 1 mg/kg), a selective adenosine A2 receptor antagonist was administered intraperitoneally (*17*). These agonists, 8-sPT, DPCPX and DMPX, were purchased from the Sigma-Aldrich Corporation (St. Louis, USA), and dilazep was donated by Kowa Co., Ltd.

Effect of Dilazep

In additional experiments, 300 μg/kg of dilazep was intravenously administered to two Japanese white rabbits to confirm its effects on adenosine concentration. Blood samples were taken before and 8 min after dilazep administration from the right femoral artery. Adenosine concentrations were measured by high performance liquid chromatography (*18*, *19*). To compare the increases in adenosine and the doses of dilazep, 150 μg/kg of dipyridamole chloride was intravenously administered in the manner described above. Rabbits were chosen for the intra-arterial adenosine concentration measurement since the effect of blood extraction for sampling to systemic circulation is negligible comparing the effect if we extract blood from a smaller animal, such as a rat.

Statistics

Data are expressed as the means±SEM. StatView software (SAS Institute Inc., Cary, USA) was used to perform all statistical analyses. Haemodynamic data (mean arterial blood pressure and left renal arterial flow) were compared by paired *t*-test as long as there were paired data, and otherwise by unpaired *t*-test. Other data were compared with the saline control group or the 300 μg/kg dilazep group for adenosine

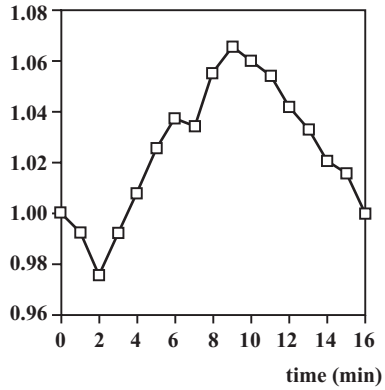

Fig. 5. *Representative time course of afferent arteriolar diameter change after dilazep administration. The changes in diameter are expressed as ratios. The afferent arteriole constricted within 2 min after administration of 300 μg/kg dilazep and then began to dilate. Vascular changes reverted to the baseline within 20 min.*

blockade by two-way ANOVA with repeated-measures. For comparison of time course differences within a group in the blockade study, *t*-tests were performed when we found a time effect was significant. A *p* value less than 0.05 was considered to be statistically significant.

Results

Haemodynamics

Table 1 shows the body weights of the rats and the basal haemodynamic data. The pattern of changes in left renal arterial flow (Fig. 2) was similar to that in glomerular arteriolar diameter following dilazep administration. A1 blockade resulted in renal arterial flow increase at the early phase and A2 blockade resulted in renal arterial flow decrease throughout the observation period, especially at the early phase (Fig. 3). Mean arterial pressure did not change throughout the experiment.

Dose of Dilazep and Observation Time

The acute effects of 10 and 100 μg/kg of dilazep were examined. Vascular diameter changes by 10 μg/kg of dilazep was unclear and only vasodilatation was observed by 100 μg/kg of dilazep without early vasoconstriction. Early vasoconstriction was observed with only 300 μg/kg of dilazep, indicating a dose dependency of dilazep (Fig. 4). Vascular changes reverted to the baseline within 20 min (Fig. 5). Based on this finding, we adopted the 10 min observation time in this study.

Visualisation of Afferent and Efferent Arterioles and the Effect of Dilazep on Them

A representative image of pre- and postglomerular arterioles as well as a glomerulus is shown in Fig. 6. Early vasoconstriction and late vasodilatation are observed. On the whole, the afferent arteriolar diameter was slightly larger than the efferent arteriolar diameter (15.7±0.7 μm, 14.0±0.7 μm, $p<0.001$). All the diameter changes are expressed as a ratio in the figures. No significant changes in diameter were observed following saline administration (Fig. 7). Following 300 μg/kg administration, we found remarkable diameter changes. One minute after administration of 300 μg/kg dilazep, both the afferent and efferent arterioles constricted by about 5% (*t*-

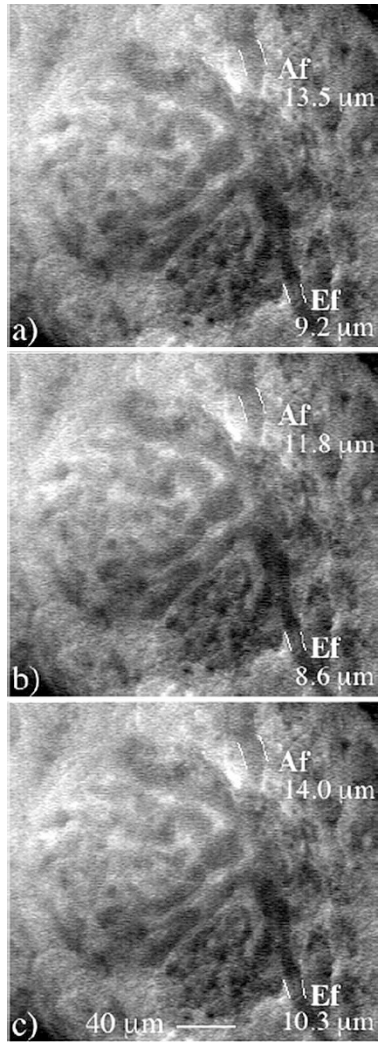

Fig. 6. *Representative images of biphasic afferent and efferent diameter change after dilazep. a: Baseline state. Af 13.5 μm, Ef 9.2 μm. b: One minute after administration of dilazep (300 μg/kg) intravenously, both afferent and efferent arterioles showed vasoconstriction. Af 11.8 μm, Ef 8.6 μm. c: Eight minutes after dilazep administration, dilatation of both afferent and efferent arterioles was recognisable. Af 14.0 μm, Ef 10.3 μm. Bar indicates 40 μm length. Af, afferent arteriole; Ef, efferent arteriole.*

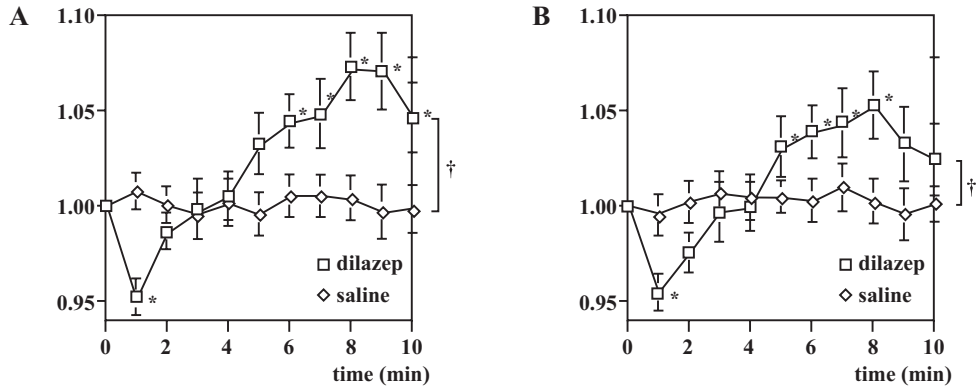

Fig. 7. *Effects of dilazep on afferent (A) and efferent arterioles (B). The changes in diameter are expressed as ratios. Biphasic afferent and efferent arteriolar diameter changes with early vasoconstriction and late vasodilatation were observed following administration of 300 μg/kg dilazep. Open square, 300 μg/kg; open diamond, saline; †$p < 0.05$ vs. saline (ANOVA), *$p < 0.05$ vs. basal (t-test).*

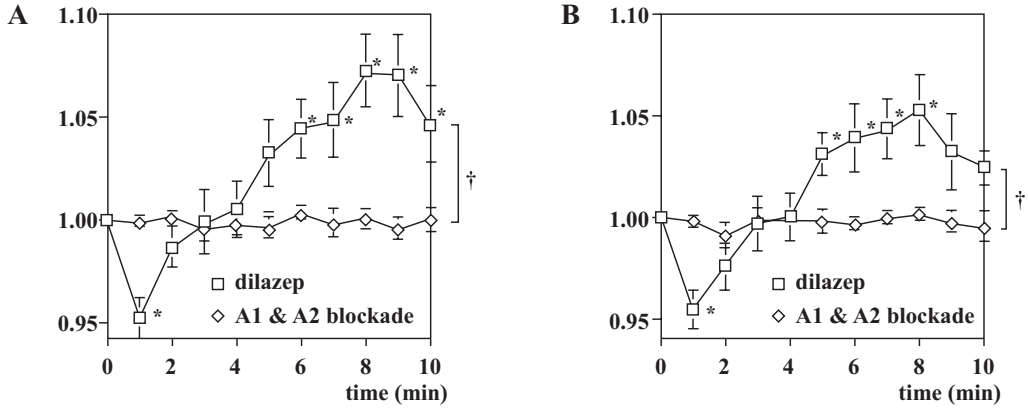

Fig. 8. *Effects of A1 and A2 receptor on afferent (A) and efferent arterioles (B) compared with dilazep. The changes in diameter are expressed as ratios. Non-specific adenosine receptor blockade (A1 and A2 blockade) by 8-p-sulfophenyl theophylline (8-sPT) abolished any afferent or efferent diameter changes, which were well observed by dilazep administration. Open square, 300 μg/kg of dilazep; open diamond, A1 and A2 receptor blockade by 8-sPT (10 mg/kg); †$p < 0.05$ vs. A1 and A2 receptor blockade (ANOVA), *$p < 0.05$ vs. basal (t-test).*

test, $p < 0.01$) and then they reverted to and exceeded the basal values and reached the maximum dilatation of 5% to 7% in around 8 min (*t*-test, $p < 0.01$, Fig. 7). Thus, early vasoconstriction was followed by vasodilatation (ANOVA, $p < 0.05$) to nearly the same degree for both afferent and efferent arterioles, with slightly larger changes for afferent arterioles.

Effect of Adenosine Receptor Antagonists on Vascular Changes

A nonselective adenosine receptor antagonist, 8-sPT, abolished both early- and late-phase diameter changes without any difference from the saline control ($p < 0.05$, Fig. 8). A selective A1 receptor antagonist, DPCPX, abolished vasoconstriction and caused a gradual increase at the early phase compared with 300 μg/kg dilazep administration ($p < 0.05$, Fig. 9). DPCPX increased the vasodilatation of both afferent and efferent arterioles in the later phase to a similar degree by about 8% in around 8 min. Note that vasodilatation occurred immediately after administration of dilazep and its degree is augmented compared with the vascular diameter changes after administration of 300 μg/kg dilazep alone. A selective adenosine A2 receptor antagonist, DMPX, completely abolished vasodilatation at the late phase with an increase in vasoconstriction in both the afferent and efferent arterioles at the early phase ($p < 0.05$, Fig. 10). Vasoconstriction continued for 10 min after administration of dilazep, and its degree was augmented compared with the vascular diameter changes

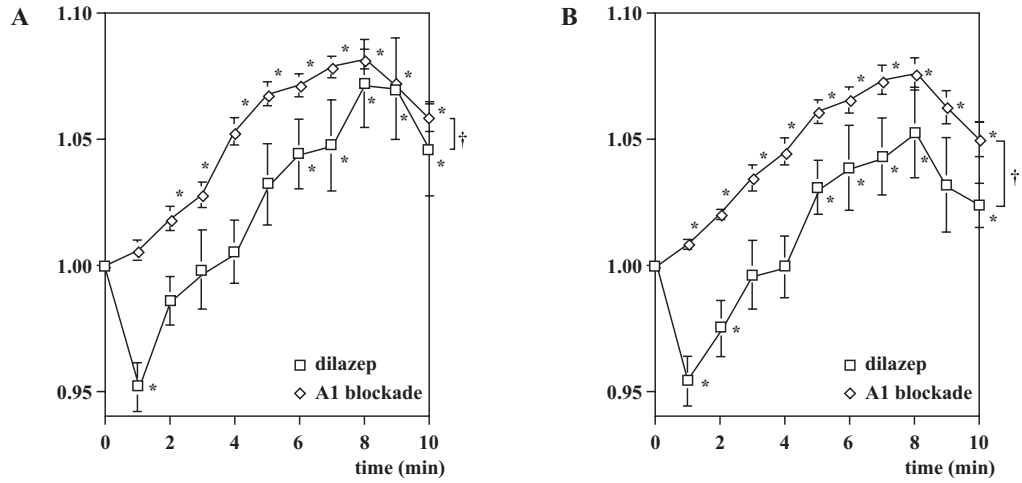

Fig. 9. *Effects of A1 receptor on afferent (A) and efferent arterioles (B). The changes in diameter are expressed as ratios. Pretreatment with a selective A1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1 mg/kg) abolished vasoconstriction by dilazep (300 μg/kg) at the early phase but enhanced the late vasodilatation. Open square, 300 μg/kg of dilazep; open diamond, A1 receptor blockade by DPCPX (1 mg/kg); †$p < 0.05$ vs. 300 μg/kg of dilazep (ANOVA), *$p < 0.05$ vs. basal (t-test).*

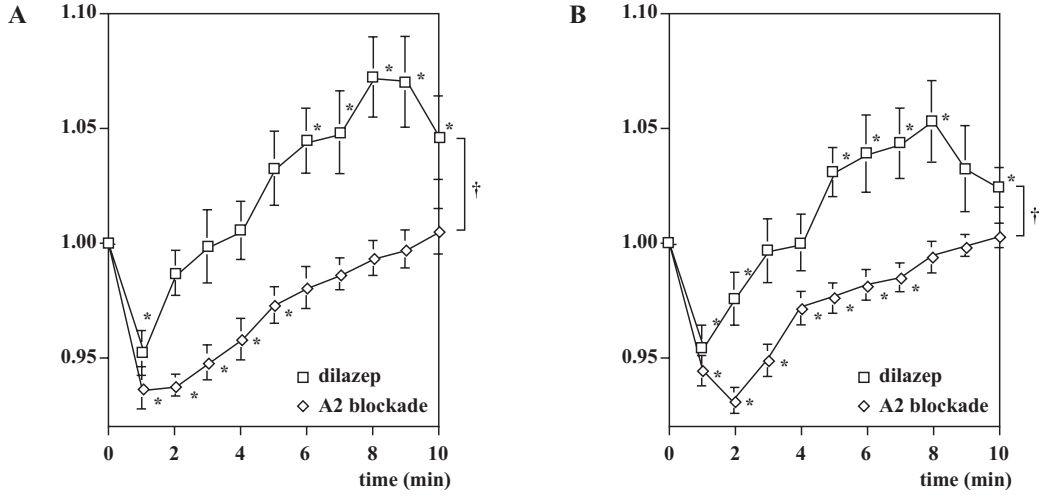

Fig. 10. *Effects of A2 receptor on afferent (A) and efferent arterioles (B). The changes in diameter are expressed as ratios. Pretreatment by a selective adenosine A2 receptor antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX, 1 mg/kg), abolished vasodilatation by dilazep (300 μg/kg) at the later phase but enhanced vasoconstriction at the early phase. Open square, 300 μg/kg of dilazep; open diamond, A2 receptor blockade by DMPX (1 mg/kg); †$p < 0.05$ vs. 300 μg/kg of dilazep (ANOVA), *$p < 0.05$ vs. basal (t-test).*

after administration of 300 μg/kg dilazep alone. The degree of vasoconstriction at the early phase was the same between the afferent and efferent arterioles but with shorter time to the maximum constriction. A1 or A2 receptor blockade made vasodilatation or vasoconstriction greater, respectively, suggesting a masking effect of the action of each receptor (Figs. 8 and 9).

Evaluation of the Intra-arterial Concentration of Adenosine after Administration of Dilazep and Dipyridamole

The intra-arterial concentration of adenosine was elevated 3-fold by intravenous administration of 300 μg/kg dilazep, from 21.0 (21 and 21 nmol/L) to 63.0 nmol/L (57 and 69 nmol/L), while intravenous administration of 150 μg/kg dipyridamole (a concentration equivalent in potency to 300 μg/kg dilazep)

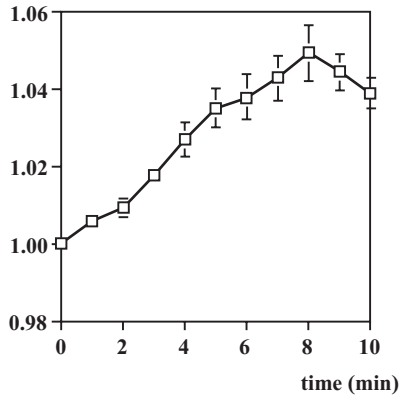

Fig. 11. *Effects of dilazep on the arteries of the mesenterium. The changes in diameter are expressed as ratios (number of rats=8). Only 5% vasodilatation was observed after administration of dilazep (300 μg/kg). The time course of the vascular diameter changes was similar to the results in the A1 blockade study (see also Fig. 9).*

increased the adenosine concentration 1.8-fold, from 23.5 (17 and 30 nmol/L) to 42.0 nmol/L (31 and 53 nmol/L) (number of rabbits=2). These adenosine concentrations (20 to 60 nmol/L) were within the physiologically effective range (*20*).

Discussion

We directly visualised the glomerular microcirculation and examined the adenosine effects augmented by dilazep on the afferent and efferent arterioles in normal Wistar rats. To our knowledge, this is the first physiological *in vivo* visualisation study that examined the acute vascular action of adenosine at the renal microvessel level, which was augmented by dilazep administration. Dilazep acutely constricted pre- and postglomerular arterioles at the early phase and dilated both vessels at the later phase. Renal arterial flow changes were in accordance with the vascular diameter changes in which vasoconstriction or vasodilatation was about 5%, matching the flow velocity changes of about 20% as predicted from Poiseuille's law. In an additional experiment, we examined local glomerular capillary flow velocity changes (*21*) following 300 μg/kg dilazep administration ($n=5$). Average flow velocity decreased in 1 min from 239±17 μm/s to 187±13 μm/s and then increased to 290±31 μm/s. Velocity changes are by about 20% for both glomerular arteriolar constriction and dilatation. To our knowledge, glomerular capillary blood flow velocity has never been measured in relation to adenosine. These capillary velocity changes were positively correlated with diameter changes. With both A1 and A2 receptor blockade, vasoconstriction and vasodilatation were abolished. These results indicate that pre- and postglomerular vascular diameter changes after dilazep administration were caused *via* adenosine receptors.

Dilazep inhibits the uptake and subsequent metabolism of adenosine. By dilazep, the endogenous interstitial adenosine concentration is considered to be elevated as dipyridamole (*22–25*). Nishiyama *et al*. reported that the effects of adenosine on renal haemodynamics depend on its concentration in the interstitium (*26*). Actually, the intra-arterial adenosine concentration was elevated by intravenous dilazep administration. If adenosine is administered from a peripheral route, *i.e.*, intravenously, it is eliminated within a half-life of seconds by carrier-mediated uptake, which occurs in most cell types, and subsequent metabolism by adenosine deaminase (*27*). Thus, dilazep, whose half-life is 4 h, is preferable to examine the acute local effect of adenosine.

As to the vasodilatation following vasoconstriction, the after-effects of the vasomodulation are considered to be involved in the biphasic diameter change as expected whenever there is a diameter change. The important point is that the after-effects were not pronounced. In fact, the contribution of after-effects is very small, and this was clarified in the present study. When the A2 receptor was blocked, the action of the A1 receptor and its after-effects were as shown in Fig. 10. There were some cases of overshooting due to after-effects, which appear within the range of error indicated in the figure. This means there were cases without any overshooting despite after-effects. Hence, vasodilatation in this biphasic pattern is mainly attributed to the action of A2 instead of after-effects.

Over a wide range of arterial pressures, renal auto-regulatory mechanisms maintain a relatively constant glomerular filtration rate and renal blood flow. Adenosine is in the mediation of such renal auto-regulation (*28*). It has a significant regulatory influence on human renal function (*29*), including renal blood flow, glomerular filtration rate, renin secretion, tubuloglomerular feedback, and tubular reabsorption of sodium and water (*30*). Under circumstances of enhanced oxygen demand or reduced supply, increased amounts of adenosine are formed (*31*), contributing to renal flow regulation.

Several studies have demonstrated that adenosine evokes both afferent and efferent arteriolar constriction (*29*). The response is complex because adenosine also causes afferent and efferent vasodilatation *via* A2 receptors (*32*). The presence of A2 receptors on preglomerular microvessels is less clear (*33*). Kawabata *et al*. have shown that dilazep dilated the efferent arterioles and attenuated tubuloglomerular feedback (TGF)–induced afferent arteriolar vasoconstriction in clearance and micropuncture experiments (*6*). It is unlikely that afferent arteriolar vasodilatation is flow-induced, since, in the present study, there was no difference in the course of vasodilatation between the afferent and efferent arterioles. For example, the time lag to the peak vasodilatation was the same. In a separate trial, we measured the changes in diameter of the arteries in the mesenterium under physiological conditions in rats (number of rats=8) along the protocol of this study. We found 5% vasodilatation only (Fig. 11). The time course of the vascular changes was similar to that observed in the A1

blockade study (see Fig. 9). Based on these results, we conclude that, without A1 receptors, vasoconstriction does not occur.

The pattern of vascular diameter changes was the same between the afferent and efferent arterioles of glomeruli. We think that the number, distribution and sensitivity of receptors determine the vascular reaction. In the case of A1 receptor action, the A1 dominant site is the afferent arteriole, while in the case of A2 receptor action, the distribution is similar for afferent and efferent arterioles. We interpret this as meaning that early efferent arteriolar vasoconstriction was passively induced by early afferent arteriolar vasoconstriction despite nondominant A1 receptor distribution in the efferent arterioles. As for later vasodilatation, it is most likely that vasodilatation is caused by a similar distribution of A2 receptors in the afferent and efferent arterioles. Because the information on efferent arterioles is less reliable, passive efferent arteriolar vasodilatation due to A2 receptors in afferent arterioles is also possible. As to sensitivity, it has already been reported that, on the whole, vessels of superficial nephrons are more sensitive than arterioles of juxtaglomerular nephrons (*2*). If we take up the sensitivity difference between afferent and efferent arterioles, it can explain the biphasic diameter change of both afferent and efferent arterioles, but investigation of receptor sensitivity is very difficult and we have not obtained any data on it yet.

Biphasic flow or diameter changes have been reported under conditions different from those used in the present study. Agmon *et al*. (*34*) examined topical effects of adenosine on renal blood flow, and biphasic blood flow changes in the corticomedullary vascular beds without global auto-regulation by the laser Doppler method. Tang *et al*. (*35*) and Gabriels *et al*. (*36*) reported vascular changes in the hydronephrotic rat kidney, in which intraglomerular pressure is higher than the normal level.

Biphasic diameter and flow changes may be interpreted as an acute reaction to possibly severe ischaemia or hypoxia. Blood flow to the kidneys is saved for other important organs, such as the brain or the heart, for the first few minutes followed by a recovery. Despite a renal arterial flow change, the ratio of the afferent to the efferent arteriolar diameter was fairly constant, with the same degree of vascular diameter changes. It is postulated that this phenomenon may function to sustain intraglomerular pressure in a nephroprotective manner.

Yukimura *et al*. reported that urinary output increased with dilazep (*5*). Our study points out that dilazep has a short term diuretic effect because of its vasodilatory effect in the short term, since the vasodilatation rate is almost the same between sole dilazep administration and A1 blockade pre-treatment. A1 antagonists are widely used as diuretics (*37*, *38*).

Limitations of the Study

We only observed glomeruli in the shallow layer of the cortex. This was done in order to minimize the adverse effects of operational intervention. The size of the observation aperture was 2×2 mm^2, and bleeding from the incision stopped within a few minutes. Because of this limitation, our findings may not necessarily be extensible to glomeruli in the deeper cortical or juxtaglomerular layers. Vessels of superficial nephrons are reported to be more sensitive than arterioles of juxtaglomerular nephrons (*2*).

According to the literature, the major roles of the A3 receptor to date are mediation of allergic responses, mediation of apoptosis, mediation of the cell cycles, preconditioning of the heart for ischemic injury, and so on (*39*). Most of these roles are related to the cardiovascular and the nervous systems. It appears that the kidney is not greatly affected by A3 receptor activation, or perhaps there have simply not been enough A3 receptor studies on the kidney. More studies on this topic will thus be needed in the future.

Conclusion

Adenosine augmented by dilazep constricted afferent and efferent arterioles at the early phase and dilated both arterioles at the later phase *via* A1 and A2 receptor activation, respectively, with a fairly constant ratio of afferent to efferent arteriolar diameter. The flow and flow velocity changes were positively correlated with the diameter changes. It was suggested that intraglomerular pressure is maintained in the acute phase despite biphasic flow change.

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