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Acetazolamide assisted Tc-99m MAG3 renography to assess renal blood flow reserve

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Objective: The present study examines whether or not baseline and acetazolamide (ACZ) Tc-99m MAG3 renography can assess renal blood flow reserve. ***Methods:*** Renography proceeded for 50 min after sequential injections of 370 MBq Tc-99m MAG3 for baseline renography and 10 min after a 1,000 mg injection of ACZ for ACZ renography. Effective renal plasma flow of renal cortex (cERPF) in each kidney and the percentage change in cERPF of those parameters (*Δ*ERPF) were obtained before and after the administration of ACZ in 10 subjects without hypertension or diabetes (normal group), in 10 with essential hypertension (hypertensive group) and in 10 who had Type 2 diabetes with hypertension (diabetic group). A placebo test was performed in the 10 without hypertension or diabetes using distilled water instead of ACZ (placebo group). ***Results:*** The placebo test performed in the 10 without hypertension or diabetes using distilled water instead of ACZ indicated that the parameter variance between the two types of renogram was below 3.2%. The cERPF of baseline and ACZ Tc-99m MAG3 renography and *Δ*ERPF in the normal, hypertensive and diabetic groups were 89 ± 10 and 110 ± 10 m*l*/min, 89 ± 14 and 117 ± 22 m*l*/min, 100 ± 23 and 112 ± 23 m*l*/min, respectively, and 24.5 ± 13.5%, 26.0 ± 9.7% and 12.3 ± 11.1%, respectively. The difference in the cERPF value was significant in the normal and hypertensive groups whereas this did not change in the diabetic group before or after ACZ administration. ***Conclusions:*** We suggested that the *Δ*ERPF determined by baseline and ACZ Tc-99m MAG3 renography is a useful parameter for assessing renal blood flow reserve.

Key words: acetazolamide, vasoreactivity, microangiopathy, Tc-99m MAG3 renography

INTRODUCTION

Taki et al.[1,2] reported that ACZ increases cerebral and renal blood flow in rabbits by about 20–30%, indicating that a dose of 12 mg/kg ACZ inhibits 20% of the carbonic anhydrase activity in red blood cells. Hypercapnia increases blood flow in tissues and organs, thus inducing increases in organ blood flow in animals that have inhaled CO_2 gas.[1,2] This gave rise to speculation that ACZ would increase renal and cerebral blood flow in a similar manner by inhibiting carbonic anhydrase activity resulting from CO_2 retention in the organs. An O-15 H_2O PET study by Hayashida et al.[3] showed that 1,000 mg of ACZ injected at sites of normal cerebral perfusion induces the maximal vasoreactive response of a 41% increase in cerebral blood flow after 10 min. We recently presented evidence showing that ACZ dilates the microvascular system as well as the renal arteries,[4] suggesting that it acts directly on the vascular system. Microangiopathy also has been demonstrated in patients with nondiabetic nephropathies such as hypertensive nephropathy.[5,6]

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The present study examines whether or not Tc-99m MAG3 renography can differentiate the severity of renal angiopathy in patients with Type 2 diabetes through the depletion of renal vasoreactivity after an intravenous injection of ACZ.

MATERIALS AND METHODS

Study population

All patients provided written informed consent to participate in the study using the format approved by the Human Studies Committee at the National Cardiovascular Center. We separated the participants into four groups as follows: patients without hypertension or diabetes (normal group), patients with essential hypertension (hypertensive group), patients with Type 2 diabetes and hypertension (diabetes group) and 10 without hypertension or diabetes who underwent a placebo test using distilled water instead of ACZ (placebo group). The clinical characteristics of the groups are shown in Table 1.

The clinical findings among all the four groups did not significantly differ except for hemoglobin A_{1C} (HbA_{1C}), fasting plasma glucose for the diabetes group compared with the normal and hypertensive groups, and blood pressure for the diabetes or hypertensive groups compared with the normal group. The clinical characteristics did not differ between the placebo and normal groups. Essential hypertension was mild to moderate (diastolic blood pressure <110 mmHg), and fulfilled the following criteria for hypertensive nephropathy: microalbuminuria to overt proteinuria, other end-organ evidence of long-term hypertension such as left ventricular hypertrophy, a family history of hypertension and normal renal function at the time of the onset of essential hypertension, thus excluding renal disease as the etiology of the hypertension.[7,8] Type 2 diabetics fulfilled the criteria for diabetic nephropathy, with patients having from microalbuminuria (30–300 mg/24 h) to overt proteinuria (g/24 h).[9] The four groups had normal renal function or mild renal dysfunction. All patients in the hypertensive and diabetic groups had been using antihypertensive medications such as calcium channel blockers. The therapy for the diabetes group was based on diet, sulfonylurea, and/or insulin. Hydronephrosis and renal artery stenosis in the three groups of patients were excluded by computed tomography and by measuring the velocity of renal arterial blood flow using color Doppler ultrasonography. The three groups did not differ in terms of gender, age, or body mass index.

Protocol for baseline and ACZ renography

We conjectured that the microcirculatory responses to vasoactive agents decrease depending on the severity of angiopathy in patients with diabetic or hypertensive nephropathies. We conducted baseline and ACZ (Diamox, Lederle, Ltd., Tokyo, Japan) renography using Tc-99m mercaptoacetylgycylglycylglycine (MAG3) (Daiichi Radioisotope Labs. Ltd., Tokyo, Japan) for 50 min. Patients were sequentially injected with 370 MBq Tc-99m MAG3 in the following manner: 1) Thirty minutes after drinking 300 m*l* of water, the patient was placed in the supine position. 2) Counts in the syringe containing Tc-

Table 1 Compared with clinical characteristics of placebo, normal subjects, essential hypertension and Type 2 diabetes with hypertension

	Placebo group (n = 10)	Normal group (n = 10)	Hypertension group (n = 10)	Diabetes group (n= 10)	p value*	p value**	p value***
Age (years)	62 ± 9	63 ± 8	63 ± 13	62 ± 12	0.62	0.70	0.62
Gender (M/F)	8/2	6/4	8/2	7/3	0.75	0.82	0.82
Body mass index (kg/m^2)	24.2 ± 3.7	24.6 ± 3.5	24.0 ± 4.0	24.9 ± 4.4	0.87	0.54	0.54
Habituation of cigarette (years)	20.2 ± 5.4	19.5 ± 4.2	24.8 ± 17.4	23.0 ± 21.8	0.03	0.74	0.74
Fasting plasma glucose (mg/m*l*)	86.2 ± 4.8	86.3 ± 5.6	88.8 ± 7.9	133.4 ± 39.5	0.24	<0.01	0.02
Hemoglobin A_{1C} (%)	4.9 ± 0.3	5.1 ± 0.2	5.3 ± 0.3	6.0 ± 0.8	0.47	<0.01	<0.01
Serum creatinine (mg/d*l*)	1.2 ± 0.4	1.0 ± 0.6	1.2 ± 0.4	1.3 ± 0.4	0.55	0.57	0.57
Total cholesterol (mg/d*l*)	188.2 ± 23.1	182.4 ± 22.5	199.7 ± 38.5	178.9 ± 31.0	0.24	0.20	0.20
Triglyceride (mg/d*l*)	113.2 ± 43.8	112.4 ± 44.3	114.9 ± 45.6	118.6 ± 46.6	0.86	0.76	0.76
HDL cholesterol (mg/d*l*)	43.3 ± 5.7	44.3 ± 8.2	42.6 ± 9.3	37.6 ± 6.2	0.70	0.27	0.27
Systolic blood pressure (mmHg)	110.2 ± 11.6	108.0 ± 12.4	138.8 ± 16.1[#]	136.4 ± 12.2[#]	0.02	<0.01	0.71
Diastolic blood pressure (mmHg)	64.3 ± 10.0	62.7 ± 9.7	78.6 ± 11.3[#]	73.8 ± 2.8[#]	<0.01	<0.01	0.21
Carotid IMT (mm)	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.3	0.9 ± 0.2	0.44	0.16	0.16

Value expressed as mean ± SD (%). n: number of patients. [#]: taking antihypertensive medication

Carotid IMT: carotid intima-media thickness. HDL cholesterol: high density lipoprotein cholesterol.

Normal group: normal subjects. Hypertension group: essential hypertension. Diabetes group: Type 2 diabetes with hypertension.

p value*: Normal group versus Hypertension group.

p value**: Normal group versus Diabetes group.

p value***: Hypertension group versus Diabetes group.

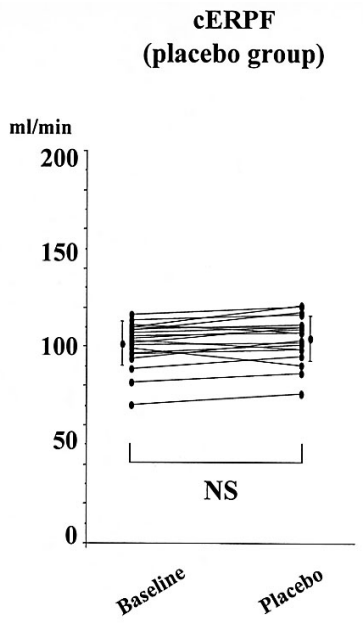

Fig. 1 Effects of placebo on renographic profiles (placebo group).

Table 2 Variation of T_{max} and cERPF in each group before and after acetazolamide (ACZ) 1,000 mg administration

	Normal group	Hypertension group	Diabetes group
		p = 0.07***	
ERPF (m*l*/min)		p = 0.16*	p = 0.07**
Baseline	89 ± 10	89 ± 14	100 ± 23
ACZ	110 ± 11[a]	117 ± 22[a]	112 ± 23

Mean values ± SD. Significantly different from baseline values, [a]p < 0.01.
*: Normal group versus Hypertension group
**: Normal group versus Diabetes group
***: Hypertension group versus Diabetes group

99m MAG3 for baseline renography were measured for 5 sec at a distance of 30 cm above the collimator of the digital gamma camera. 3) Baseline renography was performed for 20 min after the injection of 185 MBq of Tc-99m MAG3. 4) Residual counts in the syringe for baseline renography were determined. 5) Tc-99m MAG3 in the syringe for ACZ renography was counted as in step 2. 6) Ten minutes after an injection of 1000 mg ACZ dissolved in 10 m*l* distilled water, ACZ renography was performed for 20 min as in step 3 except that recording was started 1 min before the injection of 185 MBq of Tc-99m MAG3. 7) Residual counts in the syringe for ACZ renography were determined at 50 min. Renographic images were recorded over a sampling period of 10 sec for 20 min in a 128 × 128 matrix. A digital gamma camera was equipped with a high-resolution, parallel-hole collimator (Toshiba, GCA901A/HG). Results were processed using commercially available software (Toshiba, GCM5500UI) and regions of interest were delineated in both renal cortices as described.[4,10] The effective renal plasma flow of renal cortex (cERPF) in each kidney was measured using camera-based clearance techniques and Tc-99m MAG3.[4,10] Subtraction of the background from the renal activity generated time-activity curves for baseline and ACZ Tc-99m MAG3 renography from which quantitative parameters were derived. The parameters for quantitative analysis included cERPF in each kidney. The value was generated from the time activity curve of Tc-99m MAG3 in the kidney using net counts obtained by subtracting the counts in the syringe before injection from those remaining after injection.[11–13]

Assessment of vasoreactivity and reproducibility with baseline and ACZ renography

We obtained the cERPF value from ACZ renograms. We validated the vasoreactivity caused by ACZ in the 10 normal subjects, and its reproducibility using distilled water in the placebo group of 10 subjects, by calculating the percentage changes in ERPF between baseline and ACZ Tc-99m MAG3 renography as ΔERPF, using the following formula:

$$\Delta\text{ERPF} = \frac{\text{cERPF at ACZ} - \text{cERPF at baseline}}{\text{cERPF at baseline}} \times 100$$

Where "at baseline" is baseline renography and "ACZ" is ACZ renography.

Statistical analysis

The statistical significance of cERPF and ΔERPF among the four groups was evaluated by two-way analysis of variance (ANOVA) and two regression parameters were compared using a t-test. Probability values below 0.05 were considered statistically significant.

RESULTS

Variation in placebo group

The cERPF values of baseline and distilled water of the placebo group were 101 ± 11 and 104 ± 11 m*l*/min, respectively. The cERPF values of the placebo did not differ between the left and right kidneys. Variation in ΔERPF in the placebo group was 3.2 ± 4.8% (Fig. 1).

Effects of ACZ in normal, hypertensive and diabetes groups

Effects of ACZ in three groups are shown in Table 2 and Figure 2. The cERPF in each kidney of baseline and ACZ Tc-99m MAG3 renography and the ΔERPF in the normal, hypertensive and diabetic groups were 89 ± 10 and

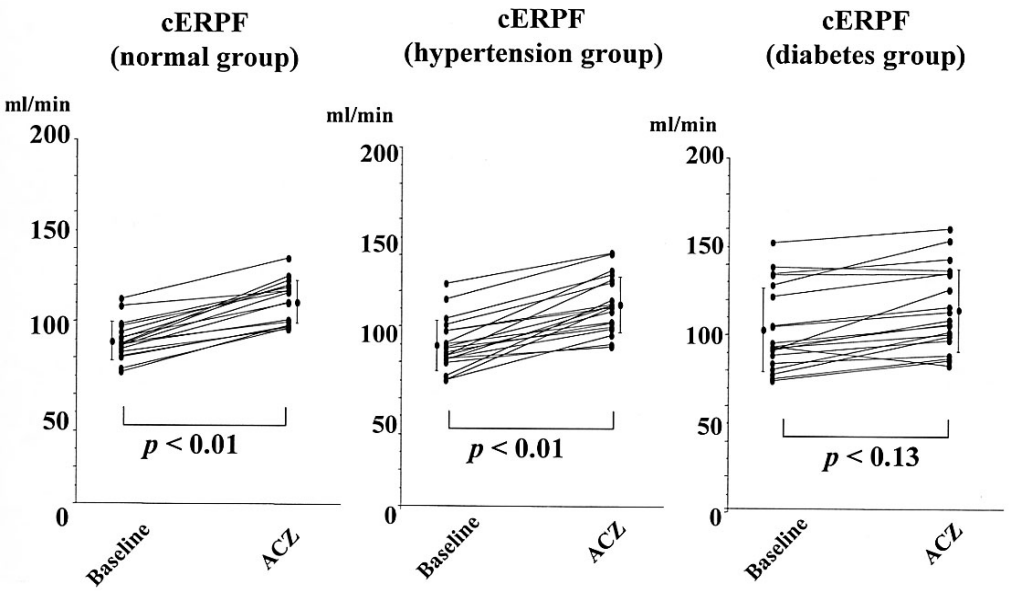

Fig. 2 Effects of acetazolamide (ACZ) in normal, hypertensive and diabetic groups. Values of ERPF significantly differ between baseline and ACZ in normal and hypertensive groups ($p < 0.01$, respectively), but those of diabetic group do not differ between baseline and ACZ ($p = 0.13$).

110 ± 10 ml/min, 89 ± 14 and 117 ± 22 ml/min, 100 ± 23 and 112 ± 23 ml/min, respectively, and 24.5 ± 13.5%, 26.0 ± 9.7% and 12.3 ± 11.1%, respectively (Fig. 3). The cERPF values of the baseline did not differ significantly among the three groups. However, the cERPF values differed significantly between baseline and ACZ in the normal and hypertensive groups ($p < 0.01$, respectively), but those of the diabetic group did not differ between baseline and ACZ.

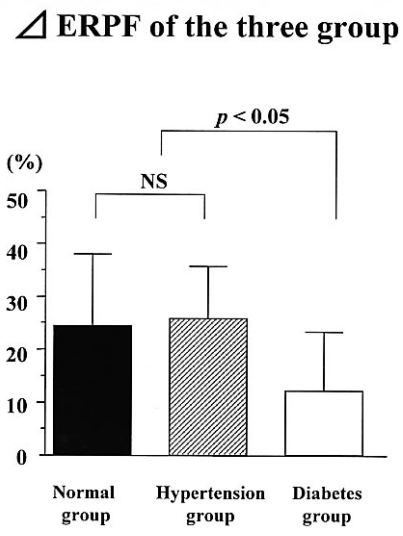

Fig. 3 Comparison of ΔERPF with baseline and ACZ renography in normal, hypertensive and diabetic groups. Values of ΔERPF in diabetic group are significantly less effective when obtained by ACZ compared with normal and hypertensive groups.

DISCUSSION

The present study examines whether or not baseline and ACZ Tc-99m MAG3 renography can assess renal blood flow reserve. In this study, the difference in the cERPF value in each kidney was significant between normal and hypertensive groups whereas they did not change in the diabetic group between before and after ACZ administration. Bosch[14] reported that the renal reserve was an indicator of the workload per nephron and might be a useful parameter to assess the progression of renal disease. Therefore, regions of interest were delineated in both renal cortices. It is suggested that the ΔERPF determined by baseline and ACZ renography is a useful parameter for assessing renal vasoreactivity in patients with Type 2 diabetes. Taki et al.[1,2] recently reported that ACZ increases cerebral and renal blood flow in rabbits by about 20–30%, indicating that a dose of 12 mg/kg ACZ inhibits 20% of the carbonic anhydrase activity in red blood cells. Their study indicated that the alterations in renal blood flow, along with several vascular factors such as prostacyclin, endothelin, and nitric oxide respond to ACZ, suggesting that the mechanism of ACZ-induced vasodilatation involves a cascade triggered by CO_2 retention similar to that induced by CO_2 inhalation.[1,2] They also revealed using colored microspheres that ACZ induces vasodilation in all vessels of the rabbit liver regardless of size, as well as in small and medium kidney vessels.[15] Several studies have examined the response to ACZ in renal blood flow, but the results were controversial. For

example, reports indicate that ACZ inhibits the absolute rate of proximal reabsorption, causes a reduction in the glomerular filtration rate and activates the tubular glomerular feedback mechanism resulting in afferent vasoconstriction in healthy humans,[16] in Type 1 diabetic patients[17–19] and in experimental models.[20,21] We recently applied ACZ to a patient with severe bilateral renovascular disease. We surmised that baseline and ACZ renography could evaluate the vasoreactivity of the renal arteries, and thus could indicate the severity of renal angiopathy, since ACZ might dilate the renal arteries.[4] Therefore, we propose that ACZ increases the renal blood flow.

Renal blood flow is maintained at a constant level by autoregulation, and the glomerular filtration rate is influenced by renal blood flow and renal vascular resistance.[22] However, Christensen et al.[23] reported that autoregulation of the glomerular filtration rate in patients with Type 2 diabetes and hypertension is often impaired. The present study found that the difference in the cERPF value in each kidney was significant between the normal and hypertensive groups, but they did not change in the diabetic group between before and after ACZ administration. The ΔERPF values were small in the diabetic group, because renal vascular resistance due to atherosclerosis might be increased compared with the normal and hypertension groups, indicating poor autoregulation of the glomerular filtration rate. This may explain why cERPF may be explained a useful parameter for baseline and ACZ renography in patients with Type 2 diabetics with hypertension. In this study, systolic and diastolic blood pressure did not change either before or after ACZ administration. Shimotsu et al.[24] also reported that ACZ does not affect blood pressure. Thus, ACZ is probably safe in patients with hypertension and Type 2 diabetes.

In addition, although previous results were obtained from single examination at least 2 days apart, all parameters concerning baseline and ACZ renography were generated from a single examination on the same day. Moreover, the present study suggested that baseline and ACZ renography can be completed within one day in short a time, and that the method is simply. The cERPF in each kidney determined using the gamma camera might be difficult to reproduce in terms of an absolute value due to kidney depth, position and attenuation. However, the present study found that the renographic variation in ΔERPF values between the baseline and the placebo did not exceed 3.2%. Therefore, we concluded that the ΔERPF value may indicate renal vasoreactivity in our method.

CONCLUSIONS

The present study examined whether or not baseline and ACZ Tc-99m MAG3 renography could assess renal blood flow reserve. The ΔERPF values of baseline and ACZ Tc-99m MAG3 renography were significantly less changed by ACZ in the diabetic than in the normal and hypertensive groups. Therefore, we suggested that the ΔERPF value determined by baseline and ACZ Tc-99m MAG3 renography is a useful parameter for assessing renal vasoreactivity in patients with Type 2 diabetes with hypertension.

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REFERENCES

1. Taki K, Hirahara K, Tomita S, Totoki T. Acetazolamide-induced increase in blood flow to rabbit organ is confirmed using colored microspheres. *Heart Vessels* 1998; 13: 63–67.
2. Taki K, Kato H, Endo S, Inada K, Totsuka K. Cascade of acetazolamide-induced vasodilatation. *Res Commun Mol Pathol Pharmacol* 1999; 103: 240–248.
3. Hayashida K, Tanaka Y, Hirose Y, Kume N, Iwata T, Miyake Y, et al. Vasoreactive effect of acetazolamide as a function of time with sequential PET ^{15}O-water measurement. *Nucl Med Commun* 1996; 17: 1047–1051.
4. Horita Y, Hayashida K, Takishita S, Kohno S, Kawano Y. Dilatation of renal artery distal to stenosis demonstrated using acetazolamide Tc-99m MAG3 scintigraphy. *Clin Nucl Med* 2001; 26: 795–796.
5. Helmchen U, Wenzel UO. Benign and malignant nephrosclerosis and renovascular disease. In: *Renal pathology: with clinical and functional correlations*, Tisher CC, Brenner BM (eds), Philadelphia; JB Lippincott Company, 1994: 1201–1236.
6. Williams PS, Foss G, Bone JM. Renal pathology and proteinuria determine progression in untreated mild/moderate chronic renal failure. *Q J Med* 1988; 252: 343–354.
7. Bianchi S, Bigazzi R, Campese VM. Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications. *Am J Kidney Dis* 1999; 34: 973–995.
8. Parving HH, Mogensen CE, Jensen HE, Evering PE. Increased urinary albumin excretion rate in benign essential hypertension. *Lancet* 1974; 1: 1190–1192.
9. Mogensen CE, Keane WF, Bennett PH, Jerums G, Parving HH, Passa P, et al. Prevention of diabetic renal disease with special reference to microalbuminuria. *Lancet* 1995; 21: 1080–1084.
10. Horita Y, Hayashida K, Inenaga T, Fukuchi K, Kohno S, Kawano Y. Renal vascular reserve could stratify angiopathy in diabetes using the baseline and acetazolamide renography with ^{99m}Tc MAG3 [Abstract]. *J Nucl Med* 2001; 42 (Suppl): p41.
11. Taylor A Jr, Manatunga A, Morton K, Reese L, Prato F, Greenberg E, et al. Multicenter trial validation of a camera-based method to measure Tc-99m mercaptoacetyltriglycine,

or Tc-99m MAG3, clearance. *Radiology* 1997; 204: 47–54.
12. Itoh K, Nonomura K, Yamashita T, Kanegae K, Murakumo M, Koyanagi T, et al. Quantification of renal function with a count-based gamma camera method using Technetium-99m-MAG3 in children. *J Nucl Med* 1996; 37: 71–75.
13. Inoue Y, Ohtake T, Yokoyama I, Yoshikawa K, Asai S, Ohtake K. Evaluation of renal function from ^{99m}Tc-MAG3 renography without blood sampling. *J Nucl Med* 1999; 40: 793–798.
14. Bosch JP. Renal reserve: a functional view of glomerular filtration rate. *Semin Nephrol* 1995; 15: 381–385.
15. Taki K, Oogoshi K, Hirahara K, Gai X, Nagashima F, Tozuka K. Preferential acetazolamide-induced vasodilation based on vessel size and organ: confirmation of peripheral vasodilation with use of colored microspheres. *Angiology* 2001; 52: 483–488.
16. Skott P, Hommel E, Bruun NB, Arnord-Larsen S, Parving HH. Effects of acetazolamide on kidney function in Type 1 (insulin-dependent) diabetic patients with diabetic nephropathy. *Diabetologia* 1988; 31: 806–810.
17. Hannedouche T, Lazaro M, Delgado AG, Boitard C, Lacour B, Grunfeld J-P. Feedback-mediated reduction in glomerular filtration during acetazolamide infusion in insulin-dependent diabetic patients. *Clin Sci* 1991; 81: 457–464.
18. Slomowitz LA, Bergamo R, Hirschberg R, Grosvenor M, Kopple JD. Enalapril attenuates the renal hemodynamic effect of acetazolamide in patients with diabetes mellitus: possible implications for tubuloglomerular feedback. *Am J Nephrol* 1996; 16: 315–319.
19. Tucker BJ, Steiner RW, Gushwa LC, Blantz RC. Studies on the tubular-glomerular feedback system in the rat. The mechanism of reduction in filtration rate with benzolamide. *J Clin Invest* 1978; 62: 993–1004.
20. Leyssac PP, Karlsen FM, Skott O. Dynamics of intrarenal pressures and glomerular filtration rate after acetazolamide. *Am J Physiol* 1991; 266: R1544–R1550.
21. Leyssac PP, Karlsen FM, Holstein-Rathlou NH, Skott O. On determinants of glomerular filtration rate after inhibition of proximal tubular reabsorption. *Am J Physiol* 1991; 261: F169–F178.
22. Smith HW. Structure and function of the kidney. In: *Clinical Nephrology*, Papper S (eds), Boston, Massachusetts; Little, Brown and Company, 1978: 35–90.
23. Christensen PK, Hansen HP, Parving HH. Impaired autoregulation of GFR in hypertensive non-insulin dependent diabetic patients. *Kidney Int* 1997; 52: 1369–1374.
24. Shimotsu Y, Hayashida K, Kume N, Fukuchi K, Nishimura T. Acetazolamide induced myocardial ischemia in patients with severe coronary artery disease. *Ann Nucl Med* 1997; 12: 21–27.