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Protective role of γ-aminobutyric acid against chronic renal failure in rats

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Abstract

The protective effect of γ-aminobutyric acid (GABA) against chronic renal failure (CRF) was investigated using a remnant kidney model with 5/6 nephrectomized rats. Nephrectomy led to renal dysfunction, which was evaluated via several parameters including serum urea nitrogen, creatinine (Cr) and Cr clearance. However, the administration of GABA ameliorated renal dysfunction, and a longer administration period of GABA increased its protective effect. In addition, nephrectomized control rats showed an elevation in the fractional excretion of sodium (FE_{Na}) with an increase in urinary sodium, while GABA led to a significant decline in FE_{Na}. Moreover, nephrectomy resulted in a decrease of serum albumin and an increase of urinary protein with a change in the urinary protein pattern, whereas the rats administered GABA showed improvement in these changes associated with CRF caused by nephrectomy. This suggests that GABA would inhibit the disease progression and have a protective role against CRF. As one of the risk factors for CRF progression, hypertension was also regulated by GABA. The results also indicate that GABA may play a protective role against CRF through improvement of the serum lipid profile, with reductions in triglyceride and total cholesterol. Furthermore, nephrectomy led to renal oxidative stress with a decrease in the activity of antioxidative enzymes and elevation of lipid peroxidation. The administration of GABA attenuated oxidative stress induced by nephrectomy through an increase in superoxide dismutase and catalase, and decrease in lipid peroxidation. The histopathological lesions, including glomerular, tubular and interstitial lesions, under nephrectomy were also improved by GABA with the inhibition of fibronectin expression. This study demonstrated that GABA attenuated renal dysfunction via regulation of blood pressure and lipid profile, and it also ameliorated the oxidative stress induced by nephrectomy, suggesting the promising potential of GABA in protecting against renal failure progression.

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Introduction

Chronic renal failure (CRF) is characterized as a pathophysiological condition of the kidney due to the permanent loss of nephrons, leading to the development of glomerular and tubular lesions. Although angiotensin-converting enzyme inhibitors, protein restriction, dialysis and transplantation are effectively employed for the management of renal disease, the number of patients with CRF, especially those undergoing dialysis and with end-stage renal diseases, is growing worldwide (Chertow et al 1996). Since progression to CRF is associated with multiple factors such as hypertension, hyperlipidaemia and oxidative stress (Sanaka et al 1991; Nihei et al 2001; Imai et al 2003; Vaziri & Liang 2004), therapeutic advances and new approaches have to be considered to prevent progression to the associated complications, as well as CRF, through the manipulation of risk factors.

γ-Aminobutyric acid (GABA), one of the major inhibitory neurotransmitters, plays an important role in cardiovascular regulation (DeFeudis 1983; Gillis et al 1984). Moreover, intracerebroventricular administration of GABA or its analogues induces a hypotensive effect, and elevation of GABA levels in the brain has also been reported to reduce blood pressure (Antonaccio et al 1978; Antonaccio & Snyder 1981; Matheson et al 1986). Furthermore, Tatsuta et al (1990) have reported that GABA inhibits gastric carcinogenesis via the $GABA_B$ receptor, and recent studies have demonstrated that GABA delays or inhibits invasion and metastasis of various types of cancer cells, such as mammary gland, colon, and hepatic

cancer cells (Kleinrok et al 1998; Minuk 2000; Opolski et al 2000).

The kidney also contains significant levels of GABA, and specific binding sites for GABA have been confirmed in the kidney (Goodyer et al 1980, 1982; Amenta et al 1988; Erdo 1990). Patients with end-stage renal disease and dialysis encephalopathy showed reductions in GABA levels in many areas of their brains, especially in the cerebral cortex and thalamus (Perry et al 1985). Furthermore, it has been suggested that GABA can modulate renal function (Monasterolo et al 1996), but there have been few reports on the effects of GABA administration in renal failure. Therefore, in this study, the effect of GABA administration was investigated using the CRF model of 5/6 nephrectomized rats.

Materials and Methods

Materials

GABA was supplied by Pharma Foods International Co. (Kyoto, Japan).

Animals and treatment

The guidelines for Animal Experimentation, approved by the University of Toyama, were followed in these experiments. Male Wistar rats, about 200 g, underwent resection of two-thirds of the left kidney and, 10–14 days later, total excision of the right kidney (Platt et al 1952; Morrison 1966). Blood urea nitrogen levels and body weight were determined after recovery from the operation, and the rats were then divided into three groups of ten (n = 10/group). There were no significant differences in blood urea nitrogen levels among the three groups. A further group of five was classified as the normal group (n = 5 per normal). One surgical group and the normal control group were given water and the other two groups were given GABA orally at a dose of 100 or 500 mg kg^{-1} daily via a stomach tube for 100 consecutive days. After induction of anaesthesia by intraperitoneal administration of nembutal (50 mg kg^{-1}), blood samples were obtained by cardiac puncture and the serum was separated immediately by centrifugation. The kidneys were subsequently extirpated from each rat following perfusion of ice-cold physiological saline through the renal artery, and then one part was immersed in formalin for histological analysis and the other part was kept at −80 °C until enzyme assay.

Serum and urine component levels

Serum urea nitrogen and creatinine (Cr), urinary protein excretion and urinary Cr were determined every 30 days until the end of the administration period. Urine samples were collected every 30 days from rats placed individually in metabolic cages. Food and water intakes and urinary volume were measured for 24 h. The serum levels of total protein, albumin, triglyceride and total cholesterol were measured at 100 days after nephrectomy. Serum and urine biochemical parameters were determined using commercial reagents (BUN Kainos and CRE-EN Kainos from Kainos Laboratories, Inc., Tokyo, Japan; A/G B-Test Wako, Triglyceride E-Test Wako and Cholesterol E-Test Wako from Wako Pure Chemical Industries, Ltd, Osaka, Japan). Urinary protein excretion was determined by the sulfosalicylic acid method (Sakagishi 1968). Cr clearance (C_{Cr}) was calculated on the basis of urinary Cr, serum Cr, urine volume and body weight using equation 1:

$$C_{Cr}\ (\text{mL/kg body weight/min}) = \{[\text{urinary Cr (mg dL}^{-1}) \times \text{urine volume (mL)}]/\text{serum Cr (mg dL}^{-1})\} \times [1000/\text{body weight (g)}] \times [1/1440\ (\text{min})] \quad (1)$$

Blood pressure

Blood pressure was measured at 25, 50 and 100 days after nephrectomy. Rats were warmed in a hot box at 37°C for 10 min, and then placed in a restraining apparatus that was also kept at 37°C. The tail was inserted through a cuff. The systolic and diastolic blood pressures were measured by the tail-cuff occlusion method (Model MK-2000, BP MONITOR FOR MICE and RATS; Muromachi Kikai, Tokyo, Japan). The average value of five measurements was used in this study.

Fractional excretion of sodium (FE_{Na})

Serum Na was measured with an electrolyte analyser (NOVA [CRT] 8; NOVA biomedical, USA), and urinary Na was measured with an electrolyte analyser (EML105; Radiometer Medical A/S, Copenhagen, Denmark) using a hydrogen electrode osmometer with the cryoscopic method. FE_{Na} was calculated on the basis of urinary Na, serum Na, urinary Cr and serum Cr, using equation 2:

$$FE_{Na}\ (\%) = [(\text{urinary Na/serum Na})/(\text{urinary Cr/serum Cr})] \times 100 \quad (2)$$

Urinary protein analysis

Urinary protein was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% polyacrylamide gel and the fractionated protein expression was analysed by a densitometric analysis NIH image system. The fractionated protein level was calculated by the ratio of each urinary protein to the total protein, and the urinary albumin level was estimated by that between urinary albumin intensity and total protein intensity. Total protein intensity was calculated as the sum of each protein intensity.

Enzyme assays

The kidney was homogenized with a 9-fold volume of iced physiological saline, and the activity of enzymes in the homogenate was determined. Superoxide dismutase (SOD) activity was assayed by the nitrous acid method (Elstner & Heupel 1976; Oyanagui 1984). Catalase activity was determined from the decrease in the amount of hydrogen peroxide (H_2O_2) (Aebi 1974). The activity of glutathione peroxidase (GSH-Px) was determined by colorimetry of 2-nitro-5-thiobenzoic acid, a compound produced by the reaction between glutathione and 5,5′-dithiobis(2-nitrobenzoic acid) (Hafeman et al 1974). Protein levels were determined according to the method of Itzhaki & Gill (1964) with bovine serum albumin as the standard.

Thiobarbituric acid (TBA)-reactive substance levels

Serum TBA-reactive substance was measured by the method of Naito & Yamanaka (1978). Renal TBA-reactive substance was assayed according to the method of Mihara & Uchiyama (1978). Briefly, the tissue was homogenized with a 9-fold volume of ice-cold physiological saline. Mitochondria were prepared from kidney homogenate by differential centrifugation (800 g and 12 000 *g*, respectively) in a refrigerated centrifuge (4°C) according to the methods of Johnson & Lardy (1967) and Jung & Pergande (1985), respectively. The pellet was resuspended in preparation medium. A sample of homogenate or pellet suspension was mixed with 1% H_3PO_4 and 0.67% TBA, and boiled for 45 min. After cooling in ice water, the reaction mixture was extracted with *n*-BuOH. The absorbance of the *n*-BuOH phase was measured at 535 nm and 520 nm. The protein level was determined by the method of Itzhaki & Gill (1964) with bovine serum albumin as the standard.

Fibronectin expression

The kidney was thoroughly homogenized with lysis buffer (250 mM NaCl, 25 mM Tris-Cl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol and 1% protein inhibitor cocktail). Homogenates were centrifuged at 4000 *g* for 15 min at 4°C and the obtained supernatants were used for Western blot analysis. The protein concentration of samples was measured using a protein assay reagent (Bio-Rad Protein Assay kit; BIO-RAD Laboratories, Richmond, CA). Samples were size-fractionated on a 4–12% gradient SDS-polyacrylamide gel and then transferred to a polyvinylidene difluoride membrane. After blocking with 5% skim milk solution for 1 h, the membrane was reacted with anti-human fibronectin antibody (dilution 1:2500) (Dako, Glostrup, Denmark) and anti-mouse β-actin antibody (dilution 1:2500) (Sigma, St Louis, MO) overnight at 4°C. Subsequently, it was incubated with horseradish peroxidase-conjugated IgG (Amersham Bioscience, Piscataway, NJ) for 90 min at room temperature and then treated with chemiluminescence reagents (Amersham Bioscience, Piscataway, NJ). The band densities were quantitated by scanning the films and analysed using the NIH Image program.

Histological observation

Renal tissues were fixed in 10% neutral-buffered formalin solution, embedded in paraffin and cut into semi-thin sections 2 μm thick. The sections were stained with haematoxylin–eosin and Masson trichrome. About 100 glomeruli in each sample were examined by light microscopy. The grade of segmental or global sclerosis was rated as grade 0–4: no change = 0, slight = 1, mild = 2, moderate = 3 or severe = 4. The degree of vascular sclerotic change and tubulointerstitial damage was also assessed according to the five grades described above.

Statistics

The results were presented as means ± s.e. values. The effect on each parameter was examined using one-way analysis of variance. Individual differences between groups were evaluated using Dunnett's test and those at $P<0.05$ were considered significant.

Results

Body and kidney weights, food and water intakes and urinary output

As shown in Table 1, the nephrectomized control rats exhibited a significant reduction in body weight but an increase in kidney weight. However, the rats administered GABA at oral doses of 100 and 500 mg kg^{-1} showed an increase in body weight compared with the nephrectomized control rats. On the other hand, no significant change in kidney weight was observed in the rats administered GABA. In the nephrectomized rats, food intake was decreased slightly, whereas water intake and urinary output significantly increased throughout the experimental period of 90 days. However, the rats administered GABA for 90 days did not show significant changes (Table 2).

Renal function

Table 2 shows the effect of GABA on parameters related to renal function under nephrectomy. The serum levels of urea nitrogen and Cr increased throughout the experimental period in nephrectomized control rats. However, the groups given GABA showed a significant decline in the levels compared with the control group. At 90 days after nephrectomy, serum urea nitrogen was elevated from 21.0 mg dL^{-1} to 73.6 mg dL^{-1}, but it was decreased to 60.0 mg dL^{-1} and 54.7 mg dL^{-1} at oral doses of GABA at 100 and 500 mg kg^{-1}, respectively. C_{Cr} was decreased by nephrectomy, whereas GABA administration significantly increased it after administration for 90 days. In addition, urinary

Table 1 Rat body and kidney weights

Group	Body weight			Kidney weight (g/100 g body weight)
	Initial (g)	Final (g)	Gain (g/100 days)	
Normal rats	306.8 ± 6.6	481.0 ± 15.7	174.2 ± 9.4	0.52 ± 0.02
Nephrectomized rats				
Control	279.6 ± 4.0[a]	415.2 ± 8.8[a]	136.3 ± 6.5[a]	0.40 ± 0.03[a]
GABA (100 mg kg^{-1} daily)	279.9 ± 4.2[a]	451.1 ± 9.7[b]	171.2 ± 9.0[b]	0.39 ± 0.02[a]
GABA (500 mg kg^{-1} daily)	279.1 ± 3.9[a]	434.1 ± 12.5[a]	155.3 ± 11.9	0.40 ± 0.03[a]

Data are means ± s.e., n = 10 rats for each nephrectomized group and 5 for normal group. [a]$P<0.05$ vs normal rats; [b]$P<0.05$ vs nephrectomized control rats.

Table 2 Rat intakes of food and water, urinary output and renal functional parameters

Group	Food intake (g/day)	Water intake (mL/day)	Urinary output (mL/day)	s-Urea nitrogen (mg dL^{-1})	s-Cr (mg dL^{-1})	Ccr (mL/kg body weight/min)	u-Protein (mg/day)
Day 30							
Normal rats	21.8 ± 0.4	49.1 ± 1.2	24.8 ± 3.0	23.9 ± 1.1	0.44 ± 0.01	5.77 ± 0.19	15.5 ± 1.9
Nephrectomized rats							
Control	18.9 ± 0.3[b]	67.8 ± 1.6[b]	42.4 ± 1.3[b]	58.2 ± 1.9[b]	0.88 ± 0.02[b]	2.85 ± 0.11[b]	37.3 ± 3.5[b]
GABA (100 mg kg^{-1} daily)	19.2 ± 0.3[b]	71.3 ± 1.7[b,c]	43.8 ± 1.2[b]	54.5 ± 1.7[b,d]	0.87 ± 0.02[b]	2.90 ± 0.10[b]	19.6 ± 2.1[e]
GABA (500 mg kg^{-1} daily)	18.8 ± 0.5[b]	71.7 ± 2.8[b,c]	45.7 ± 2.2[b,c]	53.2 ± 1.4[b,e]	0.81 ± 0.03[b,e]	3.16 ± 0.18[b,c]	19.7 ± 2.2[e]
Day 60							
Normal	21.9 ± 0.9	48.1 ± 6.0	25.6 ± 3.6	21.2 ± 0.9	0.41 ± 0.02	5.82 ± 0.15	15.9 ± 2.4
Nephrectomized rats							
Control	21.5 ± 0.5	78.3 ± 2.3[b]	50.6 ± 2.4[b]	57.5 ± 2.1[b]	1.00 ± 0.03[b]	2.83 ± 0.10[b]	70.4 ± 5.8[b]
GABA (100 mg kg^{-1} daily)	22.6 ± 0.4[d]	83.3 ± 2.1[b]	51.1 ± 2.6[b]	52.5 ± 2.7[b,d]	0.94 ± 0.02[b,d]	2.69 ± 0.08[b]	39.2 ± 5.0[b,e]
GABA (500 mg kg^{-1} daily	22.6 ± 0.8[d]	84.4 ± 3.6[b,c]	54.9 ± 3.6[b]	49.1 ± 1.5[b,e]	0.90 ± 0.03[b,e]	3.04 ± 0.10[b]	34.6 ± 5.2[b,e]
Day 90							
Normal rats	24.0 ± 0.8	53.7 ± 5.1	25.3 ± 5.3	21.0 ± 0.5	0.46 ± 0.04	5.70 ± 0.56	12.6 ± 1.7
Nephrectomized rats							
Control	20.6 ± 0.6[b]	83.7 ± 4.5[b]	53.0 ± 3.0[b]	73.6 ± 4.3[b]	1.23 ± 0.11[b]	2.16 ± 0.26[b]	67.7 ± 7.4[b]
GABA (100 mg kg^{-1} daily)	21.8 ± 0.3[b,c]	89.2 ± 1.9[b]	56.3 ± 2.6[b]	60.0 ± 2.8[b,e]	1.04 ± 0.04[b,e]	2.92 ± 0.20[b,e]	50.3 ± 3.2[b,e]
GABA (500 mg kg^{-1} daily)	22.4 ± 0.8[a,c]	84.7 ± 6.7[b]	53.2 ± 4.2[b]	54.7 ± 3.0[b,e]	0.97 ± 0.04[b,e]	2.89 ± 0.17[b,e]	48.6 ± 4.7[b,e]

Data are means ± s.e., n = 10 rats for each nephrectomized group and 5 for normal group. [a]$P < 0.01$, [b]$P < 0.001$ vs normal rats; [c]$P < 0.05$, [d]$P < 0.01$, [e]$P < 0.001$ vs nephrectomized control rats.

protein was elevated in nephrectomized rats compared with normal rats, while it was significantly decreased in rats given GABA for 30, 60 and 90 days. Renal dysfunction was more aggravated time-dependently after nephrectomy; however, GABA showed a strong protective effect against renal dysfunction in both a time- and dose-dependent manner.

Blood pressure

Figure 1 shows the effect of GABA on blood pressure under nephrectomy. The systolic and diastolic blood pressures were significantly higher in nephrectomized rats compared with normal rats. However, the systolic blood pressure of rats given GABA at doses of 100 and 500 mg kg^{-1} daily were significantly suppressed compared with nephrectomized control rats at 50 and 100 days. In addition, the diastolic blood pressures of nephrectomized rats were also significantly lowered by administration of GABA for 25, 50 and 100 days.

FE_{Na}

As shown in Figure 2, FE_{Na} was markedly elevated from 0.37% to 1.21% in nephrectomized control rats with a significant elevation in urinary sodium. However, oral administration of GABA reduced FE_{Na} significantly to 0.96% and 0.83% at a dose of 100 and 500 mg kg^{-1} daily, respectively.

Pattern of urinary protein and albumin, and serum total protein and albumin

The effects of GABA on the urinary protein pattern and urinary albumin intensity were also evaluated (Figure 3). The urinary protein pattern of nephrectomized control rats was different from that of normal rats. The bands were divided into low- and high-molecular-weight proteins relative to albumin (66 kDa). The urine of nephrectomized control rats showed low-molecular-weight protein bands, and also two bands located in the molecular weight ranges of 75–100 kDa and 100–150 kDa. The pattern in GABA-administered rats was similar to that in nephrectomized control rats; however, the intensity of low-molecular-weight protein bands was improved. Moreover, urinary albumin was markedly increased by nephrectomy, although the administration of GABA led to a decrease in urinary albumin. In contrast, the decrease in serum total protein and albumin was elevated by GABA in a dose-dependent manner (Figure 3).

Hyperlipidaemia

As shown in Figure 4 (upper panel), nephrectomy led to a significant increase in triglyceride from 62.5 mg dL^{-1} to 99.7 mg dL^{-1}, but GABA administration of 500 mg kg^{-1} decreased the level to 60.6 mg dL^{-1}. Moreover, the increased level of total cholesterol from 56.5 mg dL^{-1} to 116.9 mg dL^{-1} was decreased by a 500-mg kg^{-1} oral dose of GABA to 97.8 mg dL^{-1}.

Renal enzyme activity

Nephrectomy led to changes in the activity of reactive oxygen species-scavenging enzymes in the kidney and GABA administration affected this activity (Figure 4, middle panel). While the renal SOD activity of nephrectomized control rats was significantly lower compared with normal rats, it was increased significantly from 16.0 U (mg protein)$^{-1}$ to 26.2 U (mg protein)$^{-1}$ by oral administration of GABA at a dose of

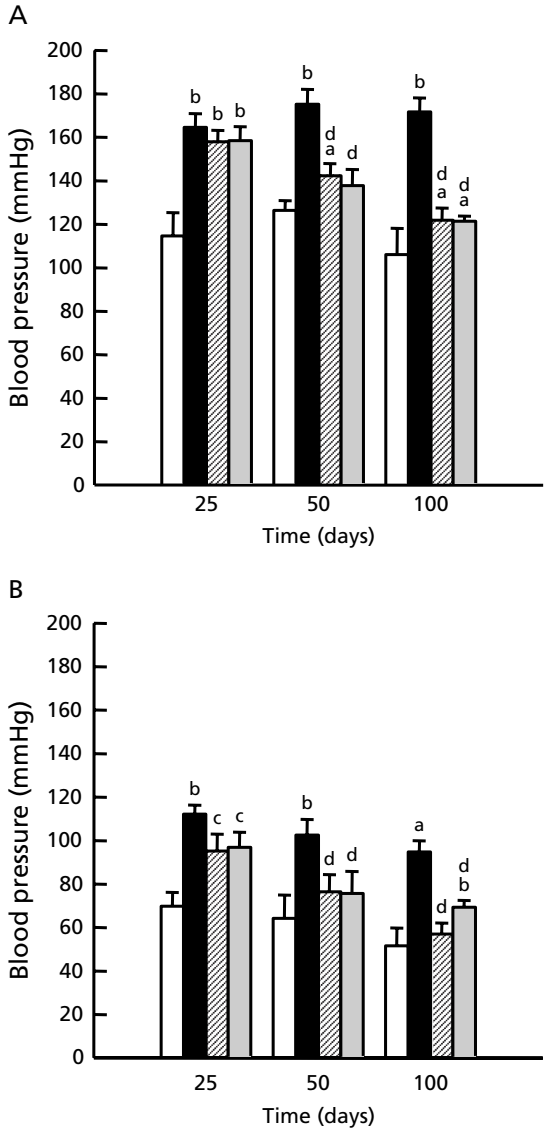

Figure 1 Systolic blood pressure (A) and diastolic blood pressure (B) in normal rats (n = 5, □) and nephrectomized rats (n = 10 per each group) treated with GABA 100 mg kg^{-1} daily (▨), 500 mg kg^{-1} daily (▩) or water (control, ■). $^{a}P < 0.01$, $^{b}P < 0.001$ vs normal rats; $^{c}P < 0.01$, $^{d}P < 0.001$ vs nephrectomized control rats.

500 mg kg^{-1} daily. In addition, the decreased activity of renal catalase in nephrectomized rats was elevated at a 500-mg kg^{-1} oral dose of GABA. On the other hand, the renal GSH-Px activity was increased in the nephrectomized control rats; however, it was significantly reduced by GABA at a 500-mg kg^{-1} dose.

TBA-reactive substance levels in the serum, renal homogenate and mitochondria

Figure 4 (lower panel) also shows the effect of GABA on the lipid peroxidation products, TBA-reactive substance levels. The serum TBA-reactive substance level was elevated in nephrectomized control rats; however, oral administration of

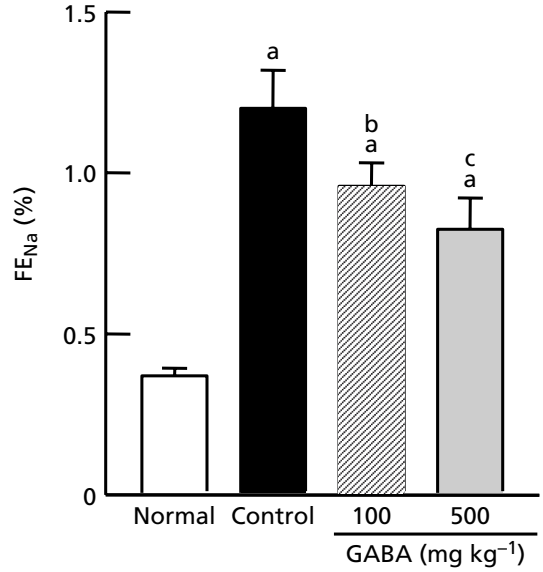

Figure 2 FE_{Na} in normal rats (n = 5, □) and nephrectomized rats (n = 10 per each group) treated with GABA 100 mg kg^{-1} daily (▨), 500 mg kg^{-1} daily (▩) or water (control, ■). $^{a}P < 0.001$ vs normal rats; $^{b}P < 0.01$, $^{c}P < 0.001$ vs nephrectomized control rats.

GABA at doses of 100 and 500 mg kg^{-1} daily led to significant decreases from 3.35 nmol mL^{-1} to 2.32 nmol mL^{-1} and 1.91 nmol mL^{-1}, respectively. Moreover, the increases in TBA-reactive substances of the renal homogenate and mitochondria in control nephrectomized rats were significantly decreased by oral administration of GABA at an oral dose of 500 mg kg^{-1} from 1.75 nmol (mg protein)$^{-1}$ to 1.15 nmol (mg protein)$^{-1}$ and from 2.04 nmol (mg protein)$^{-1}$ to 1.07 nmol (mg protein)$^{-1}$, respectively.

Fibronectin expression

As indicated in Figure 5, nephrectomy resulted in the elevation of fibronectin expression. However, the expression of fibronectin in rats administered GABA at oral doses of 100 and 500 mg kg^{-1} was reduced by approximately 30% and 55%, respectively, as compared with nephrectomized control rats.

Histological findings

The results of histopathological findings in the kidney under nephrectomy are summarized in Figure 5. The estimated renal damage via glomerular sclerosis, vascular lesion and tubulointerstitial damage was severe in nephrectomized rats, whereas the administration of GABA attenuated the renal lesions induced by nephrectomy. The score of glomerular sclerosis was reduced from 3.56 to 2.44, and that of vascular lesion was reduced from 3.22 to 2.44 at 500 mg kg^{-1} administration. Moreover, tubulointerstitial damage was also ameliorated, as shown in the score reduction from 4.00 to 2.50. Representative photomicrographs of the kidney obtained from each group are shown in Figure 5. Severe segmental or global glomerular sclerosis (or both) and tubulointerstitial

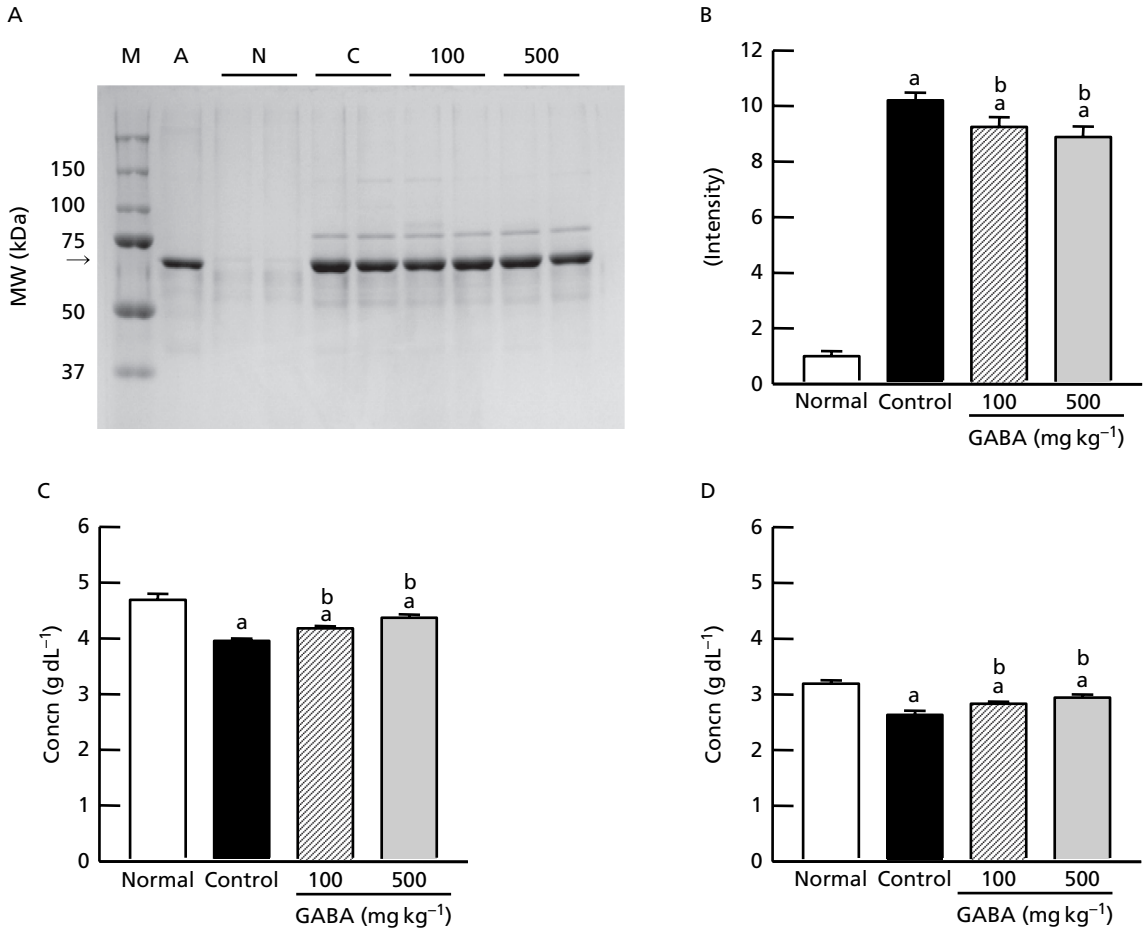

Figure 3 SDS-PAGE pattern of proteinuria (A), urinary albumin (B), serum total protein (C) and serum albumin (D) in normal rats (n = 5) and nephrectomized rats (n = 10 per each group) treated with GABA 100 mg kg^{-1} daily or 500 mg kg^{-1} daily or water (control). $^{a}P < 0.001$ vs normal rats; $^{b}P < 0.001$ vs nephrectomized control rats.

damage, such as tubular atrophy, dilatation and inflammatory cell infiltration, were observed in the nephrectomized control group; however, the rats given GABA showed less marked glomerular and tubulointerstitial damage compared with the control rats.

Discussion

Among the several theories of pathophysiology in progressive renal failure, the most convincing one suggests that the reduction in nephron number damages the remaining nephrons, which undergo hypertrophy and suffer the consequences of adaptive increases in glomerular pressure and flow that precede CRF (Hostetter et al 1981; Brenner 1985; Remuzzi et al 1997). In addition, several studies have demonstrated that nephrectomy in rats decreased the antioxidative capacity and increased oxidative stress in the remaining kidney, which aggravated CRF (Harris et al 1988; Schrier et al 1988; Sanaka et al 1991; Yokozawa et al 1997). Sener et al (2004a, b) also demonstrated that the imbalance between increased production of reactive oxygen species, as well as limited or decreased antioxidant capacity, results in the pathogenesis of CRF and it may also contribute to the development of serious complications in CRF, including hypertension, anaemia or arteriosclerosis. Based on this evidence, we employed a nephrectomy rat model to investigate the protective effect of GABA against renal failure.

The rat model with 5/6 nephrectomy led to CRF with physiological abnormalities including a decrease in body weight and increase in organ weight along with changes in food intake and urinary output. However, administration of GABA attenuated the physiological changes associated with CRF, implying that GABA normalized the physiological function to some extent. Although death has not been observed during the experimental period under the CRF rat model, renal dysfunction was severely aggravated with elevations in serum urea nitrogen and Cr levels, two important pathological parameters of renal failure. However, GABA administration ameliorated renal dysfunction, and the longer administration period of GABA, the higher its protective effect against CRF. CRF was also characterized by a subsequent decrease in C_{Cr}, an effective index of the glomerular filtration rate. Another study also demonstrated that in patients with renal failure, C_{Cr} decreases exponentially, and eventually causes

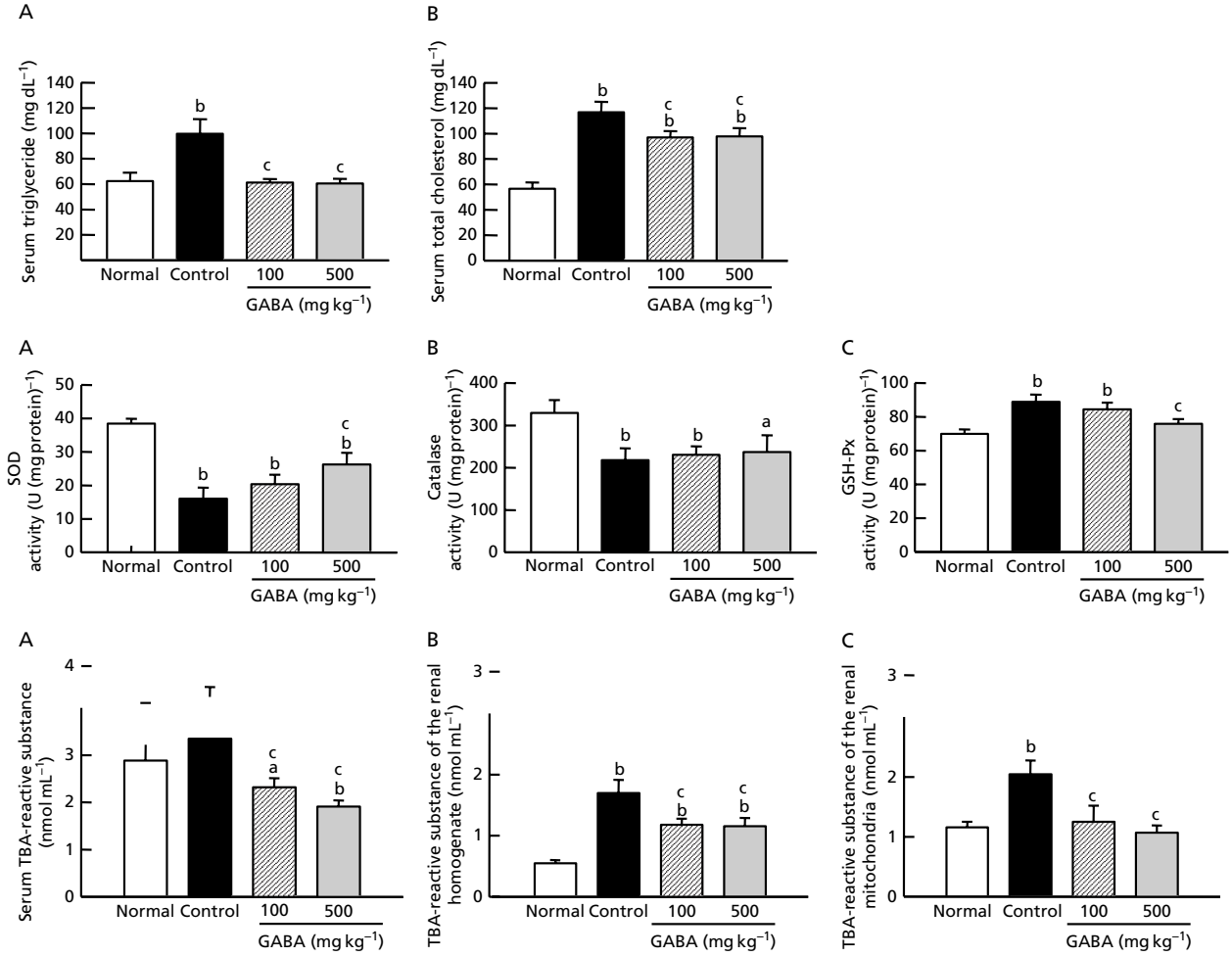

Figure 4 Serum lipids (upper panel), antioxidative enzyme activity (middle panel) and TBA-reactive substance (lower panel) in normal rats (n = 5) and nephrectomized rats (n = 10 per each group) treated with GABA 100 mg kg^{-1} daily or 500 mg kg^{-1} daily or water (control). $^{a}P < 0.01$, $^{b}P < 0.001$ vs normal rats; $^{c}P < 0.001$ vs nephrectomized control rats.

nephritic syndrome (Bell 1991). However, our study demonstrated a significant increase of C_{Cr} after administration of GABA. Therefore, it suggests that GABA can prevent or delay the progression of CRF. Furthermore, in CRF, there is an adaptive increase in sodium excretion by each functioning nephron (Slatopolsky et al 1968; Hayslett et al 1969). In this way, sodium balance can be maintained despite the diminished glomerular filtration rate without alternation in the intakes of salt and water under CRF. The mechanisms responsible for the increased FE_{Na} and marked natriuresis per nephron in the remnant kidney have previously been investigated (Hayslett et al 1969; Fine et al 1978; Trizna et al 1981). Under the CRF rat model by nephrectomy, an elevation in FE_{Na} along with an increase in urinary sodium was observed, indicating that the tubulus did not reabsorb sodium, and sodium was excreted via the urine. The increase of FE_{Na} after nephrectomy was inhibited by GABA, reflecting its amelioration of renal failure.

Hypertension is also considered as one of the risk factors for CRF progression. Since it is important not only to improve renal function but also to protect against these risk factors for progression to CRF, regulation of blood pressure under hypertension is also a crucial therapeutic approach for prevention of CRF progression. Several recent studies reported that GABA and various GABA-containing products decrease blood pressure in rat and hypertensive patients (Hayakawa et al 2002, 2004; Aoki et al 2003). In this study, GABA significantly suppressed the blood pressure in nephrectomized rats without leading to changes in normal levels. Consistent with these results, it was demonstrated that GABA lowered blood pressure only in hypertensive rats, and not in rats with normal blood pressure (Hayakawa et al 2002). The antihypertensive effect of GABA was reported to be via inhibition of noradrenaline release from sympathetic nerves in the mesenteric arterial bed via presynaptic $GABA_B$ receptors in spontaneously hypertensive rats (Hayakawa et al 2002). Although the suppression mechanism of blood pressure by GABA is still not fully clear, GABA lowers high blood pressure under hypertension. Recent study shows the multiple feedback mechanism controls both GABA concentration and inhibitory effect (Petroff 2002). GABA is considered to regulate the blood pressure for the normal range. Furthermore, GABA is also reported to be an important thermoregulatory neurotransmitter (Ishiwata et al 2005). It inhibits heat loss under cold ambient temperature, while conversely it inhibits heat production under hot temperature.

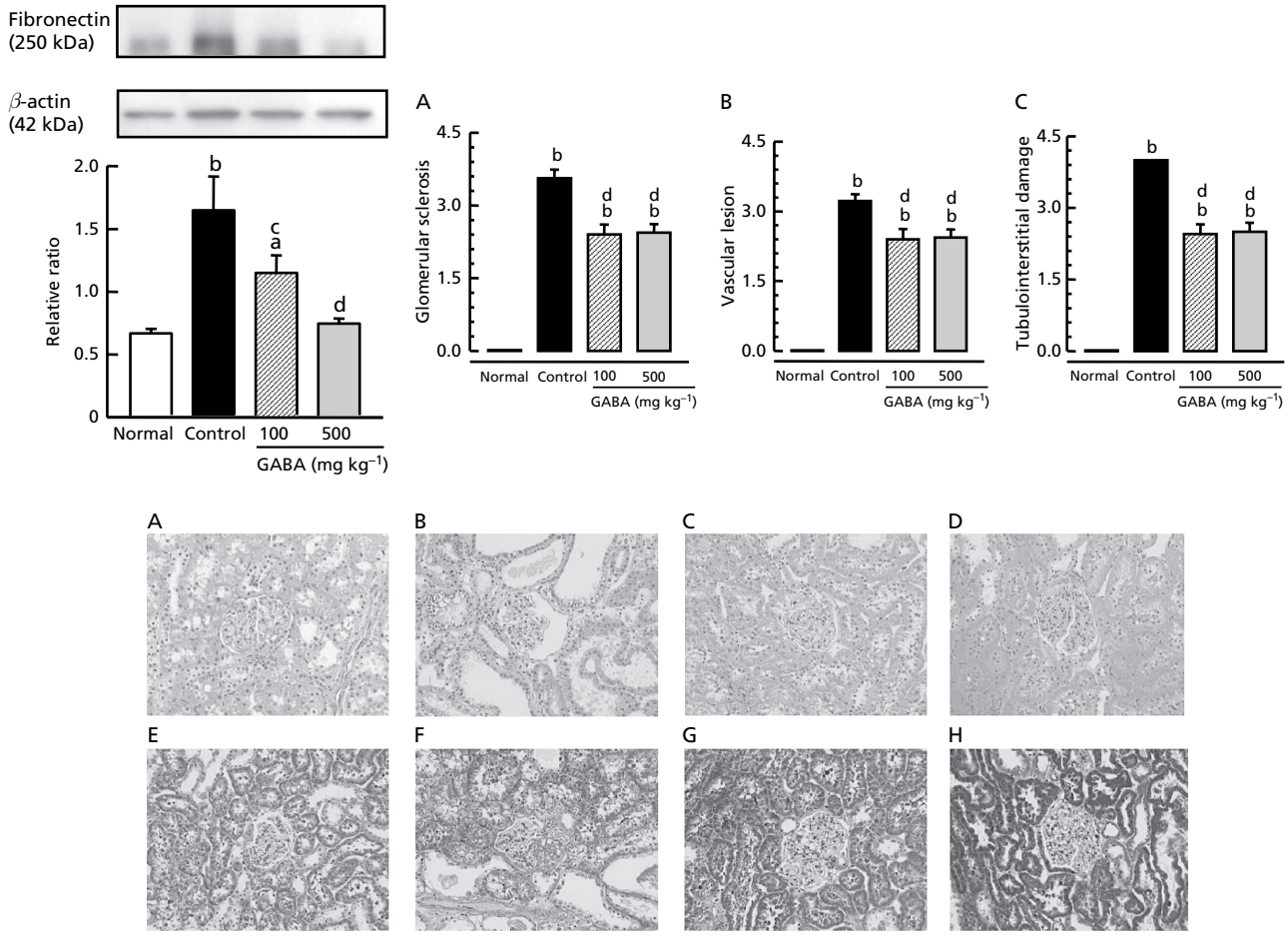

Figure 5 Western blot analysis of renal fibronectin and histopathological evaluation in normal rats (n = 5) and nephrectomized rats (n = 10 per each group) treated with GABA 100 mg kg^{-1} daily or 500 mg kg^{-1} daily or water (control), and photomicrographs of the kidney (upper panel, haematoxylin–eosin staining, ×200; lower panel, Masson trichrome staining, ×200) obtained from normal rats (A, E) and nephrectomized rats in the control (B, F) and GABA-treated (100 mg kg^{-1} daily (C, G) and 500 mg kg^{-1} daily (D, H)) groups. $^{a}P < 0.05$, $^{b}P < 0.001$ vs normal rats; $^{c}P < 0.01$, $^{d}P < 0.001$ vs nephrectomized control rats.

CRF is also associated with an increased risk of arteriosclerotic cardiovascular disease by an alteration of the plasma lipid profile (Kasiske et al 1990; Vaziri & Liang 2004). The elevated levels of serum triglyceride and cholesterol are risk factors for CRF. Our results showed that serum triglyceride and total cholesterol levels were increased under the CRF rat model. CRF results in acquired hepatic LDL-receptor-related protein deficiency, which can contribute to atherogenic diathesis and elevated plasma lipoprotein remnants (Kim & Vaziri 2005). However, the administration of GABA led to the reduction of serum levels of triglyceride and total cholesterol. This observation suggests that GABA may play a protective role against CRF through its improvement of the serum lipid profile.

It has been demonstrated that analysis of urinary proteins is very useful in the diagnosis and treatment of kidney diseases, and more recent evidence suggests that proteins filtered through the glomerular capillaries can have an intrinsic renal toxicity that might well contribute to the progression of renal damage and eventual renal failure (Hauser et al 2001). In fact, renal lesions at different kidney locations, such as glomerular and tubular areas, show typical molecular weight urinary protein patterns (Regeniter et al 2000). Usually, serum high-molecular-weight proteins, serum albumin and low-molecular-weight proteins are filtered though the glomerular capillaries and are reabsorbed in tubules. However, those proteins are present in the urine when glomeruli and tubules are damaged. Therefore, urinary albumin was used as a marker of glomerular injury. The nephrectomized rats showed glomerular lesions with an increase in urinary albumin. However, the administration of GABA led to a significant decrease in urinary albumin, although the inhibitory activity against increase in urinary albumin was relatively low as compared with other parameters related to renal failure. Since albumin is the major serum protein and acts as a carrier for a variety of nutrients, hormones and mediations, the elevation in urinary albumin under CRF indicates the blood levels of these drop as well. Other proteins that are lost in the urine include immunoglobulins, transferrin and vitamin D binding protein that are responsible for the infections, anaemia and rickets. Although further study has to be supported, the strong inhibitory effect on increase in urinary protein relative to that on urinary albumin is also

attributed to the protection from other pathological conditions. In addition, although high-molecular-weight proteins were excreted in the urine of nephrectomized rats, the pattern of protein excretion was changed by GABA administration with a reduction of high-molecular-weight protein, and albumin excretion was also decreased by the administration of GABA. Furthermore, while serum total protein and serum albumin were decreased in nephrectomized rats, the administration of GABA increased these values, implying that GABA may delay glomerular injury under CRF.

The remaining nephrons in nephrectomized rats work excessively to maintain renal function. As a result, the increased oxygen consumption in the remaining nephrons is attributable to both the decrease in antioxidative activity and the state of oxidative stress (Harris et al 1988; Schrier et al 1988). It was also reported that decreased antioxidant enzyme activity in the renal cortex of rats after nephrectomy and increased oxidative stress related to nephrectomy play a role in the development of renal fibrosis (Yokozawa et al 1997). The oral administration of GABA resulted in increased SOD and catalase activity. SOD and catalase are considered to be associated with the protective role of GABA against renal failure by nephrectomy. SOD is an upstream enzyme that protects against the deleterious actions of superoxide anion (O_2^-) by catalysing its dismutation to H_2O_2 plus oxygen. Catalase exclusively detoxifies H_2O_2 and requires no electron donor. On the other hand, the activity of GSH-Px showed an increase in nephrectomized rats. Elevation of GSH-Px activity was probably followed by a reduction of catalase activity in nephrectomized rats. However, the administration of GABA led to a decline in the activity of GSH-Px. This indicates that GABA has an influence on the $O_2^- \rightarrow H_2O_2 \rightarrow H_2O$ system catalysed by SOD and catalase. On the other hand, the ability of GSH-Px to reduce H_2O_2 or other hydroperoxide is dependent on the activity of glutathione reductase as well as the availability of NADPH and GSH (Fantone & Ward 1982). Considering this, GSH-Px not only acts as an H_2O_2 scavenger, but also works in the oxidation–reduction system. The action of GSH-Px by the administration of GABA is different from the actions of SOD and catalase, whose behaviour is mainly controlled by radical scavenging. Although verification of the protective effects of GABA against GSH-Px requires further investigations into the oxidation and reduction systems in relation to GSH and NADPH, this study clearly indicated the protective role of GABA against oxidative stress under CRF.

Oxidative stress under CRF results in elevated lipid peroxidation. Several studies have shown the elevated plasma concentrations of lipid peroxidation products in man and animals with CRF (Trznadel et al 1989; Paul et al 1991). The nephrectomized rat model also supported the increase in lipid peroxidation, indicating renal oxidative stress under CRF (Vaziri et al 2002). These results of TBA-reactive substance levels in serum, renal homogenate and mitochondria showed significant increases; however, the rats administered GABA showed significant decreases in the values. Therefore, GABA may inhibit the accumulation of lipid peroxidation products and protect renal mitochondria from oxidative stress due to nephrectomy. These findings suggest that GABA would ameliorate oxidative stress through the regulation of lipid peroxidation. Further study on the investigation of GABA effect on the time-course changes in TBA-reactive substances has to be also supported to demonstrate clearly the protective activity from oxidative stress.

The progression of CRF and renal interstitial fibrosis is related to the expression of multiple growth factors and cytokines. Fibronectin, an important component of the extracellular matrix, is a high-molecular-weight glycoprotein that mediates interactions between cells and extracellular substrates. The localization of fibronectin in the glomeruli and peritubular interstitium in renal diseases has been described (Kusano et al 2002). In our study, nephrectomy led to the induction of fibronectin expression. This result may indicate that the kidney under the CRF rat model underwent glomerulosclerosis and tubulointerstitial fibrosis. However, fibronectin expression in rats given GABA was decreased dose-dependently. In addition, clear histological evidence of the increased severity of glomerular, tubular and interstitial lesions was observed under nephrectomy, consistent with other reports (Hostetter et al 1981; Olson et al 1982; Yoshida et al 1988, 1989). In nephrectomized rats given GABA, the incidence of glomerular sclerosis, vascular lesion and tubulointerstitial damage was decreased. Therefore, GABA may retard the development of glomerulosclerosis and tubulointerstitial fibrosis.

This study demonstrates that GABA may play a protective role against CRF. GABA ameliorated renal dysfunction under CRF and regulated the hypertension induced by CRF. In particular, this study indicates that GABA ameliorated the renal oxidative stress as shown by the decrease in lipid peroxidation level and increase in antioxidative enzyme activity. The protective effect of GABA from renal dysfunction through decrease in oxidative stress would be mainly responsible for the prevention of the progression of CRF. Although the protective mechanisms of GABA against the progression of renal failure remain to be elucidated, this study suggests the promising potential of GABA for protection against renal failure.

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