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European Journal of Pharmacology 524 (2005) 120–125
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# Role of the renal nerves in γ-aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats

Kazuhito Hayakawa [a,*], Masayuki Kimura [a], Yukio Yamori [b]

[a] Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi-shi, Tokyo 186-8650, Japan
[b] International Centre for Research on Primary Prevention of Cardiovascular Diseases, 86-2 Shimobaba-cho Jodoji, Sakyo-ku, Kyoto 606-8413, Japan

Received 8 September 2005; accepted 14 September 2005
Available online 25 October 2005

**Abstract**

The aim of this study was to clarify the role of the renal sympathetic nerves in the γ-aminobutyric acid (GABA)-induced hypotensive effect in spontaneously hypertensive rats. Male spontaneously hypertensive rats (SHR/Izm) aged 7 weeks were divided into four groups on the basis of diet (containing 0.05% GABA, or GABA-free control diet) and operation (renal sympathetic-denervated or sham-operated) ($n=10$, each). Water intake, urine volume and urinary sodium were, or tended to be, slightly higher, while plasma renin activity was significantly lower in the GABA group than the GABA-free control group. GABA inhibited the development of hypertension in sham-operated spontaneously hypertensive rats but not in renal-denervated spontaneously hypertensive rats. Plasma renin activity was significantly higher in sham-operated spontaneously hypertensive rats fed the control diet than in the other three groups. These results suggest that a reduction in the effects induced by the renal nerves may play an important role in the hypotensive effect induced in spontaneously hypertensive rats by chronic dietary administration of GABA.


*Keywords:* GABA (γ-aminobutyric acid); Antihypertensive; Kidney; Spontaneously hypertensive rat; Renal denervation; Renin

## 1. Introduction

γ-Aminobutyric acid (GABA) serves as a major inhibitory neurotransmitter within the central nervous system (Curtis and Johnston, 1974), and it is also found in peripheral tissues (Jessen et al., 1979). Administration of exogenous GABA plays an important role in the modulation of cardiovascular functions (Gillis et al., 1980) by acting not only within the central nervous system but also within the peripheral tissues (Defeudis et al., 1981; Defeudis, 1982). After systemic administration, GABA reduces blood pressure immediately in both experimental animals (Takahashi et al., 1955) and human subjects (Elliott and Hobbiger, 1959). Moreover, GABA reportedly modulates vascular tone by suppressing noradrenaline release in the isolated rabbit ear artery and the pulmonary vascular bed of the cat (Manzini et al., 1985; Kaye et al., 2004) through stimulation of prejunctional GABA receptors. The mechanisms underlying the hypotensive effect of GABA have been fairly well elucidated in the central nervous system (Warder, 2001) but not fully in the periphery.

In a previous study, we confirmed that a single p.o. or i.v. administration of GABA induced a significant temporary lowering of blood pressure in spontaneously hypertensive rats but not in normotensive Wistar–Kyoto rats (Hayakawa et al., 2002, Kimura et al., 2002). We also suggested that GABA inhibits noradrenaline release through an action on presynaptic $GABA_B$ receptors and thereby suppresses the increase in perfusion pressure induced by perivascular nerve stimulation in mesenteric arterial bed isolated from spontaneously hypertensive rats (Hayakawa et al., 2002). This hypotensive mechanism may explain the short- or medium-term hypotensive effects of GABA, but it is unclear whether it is relevant to the long-term hypotensive effects observed following its chronic administration in both spontaneously hypertensive rats (Hayakawa et al., 2004) and mildly hypertensive people (Inoue et al., 2003).

It is well known that the long-term regulation of blood pressure is linked closely to renal function (Lohmeier, 2001). In the rat kidney, GABA acts on presynaptic $GABA_B$ receptors to

* Corresponding author. Tel.: +81 42 577 8971; fax: +81 42 577 3020.
*E-mail address:* kazuhito-hayakawa@yakult.co.jp (K. Hayakawa).

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doi:10.1016/j.ejphar.2005.09.020

suppress neurotransmitter release (and thereby attenuates renal vasoconstriction) (Monasterolo et al., 1996; Fujimura et al., 1999). Moreover, the renal sympathetic nerves play important roles in sodium restriction and the release of renin (Golin et al., 2001).

In the absence of reports concerning a GABA-induced hypotensive effect in spontaneously hypertensive rats, we employed bilateral renal sympathetic denervation of spontaneously hypertensive rats to clarify whether modulation of the effects of renal sympathetic nerve activity might be involved in the expression of the hypotensive effect of chronically administered GABA.

## 2. Materials and methods

We performed two series of experiments: examination of the effects of GABA on natriuresis (Experiment 1) and on spontaneously hypertensive rats subjected to bilateral renal sympathetic denervation (Experiment 2).

### 2.1. Animals and diets

Male spontaneously hypertensive rats (SHR/Izm), 6 weeks old, were purchased from Japan SLC, Inc. (Shizuoka, Japan), the breeder for the Disease Model Cooperative Research Association (Kyoto, Japan). The experimental diets (GABA-free control and GABA-containing diets) were based on the AIN-93G purified diet formulation (Reeves et al., 1993), with 0.5 g GABA (Sigma, St. Louis, MO, USA) being added to each kilogram of the base formulation to create the GABA-containing experimental diet. All animals were fed on the original AIN-93G purified diet for 3 or 4 weeks (rats aged 6–8 (Exp. 1) or 6–9 (Exp. 2) weeks), then on either that diet or the GABA-containing diet for 4 or 3 weeks (rats aged 8–12 (Exp. 1) or 9–12 (Exp. 2) weeks). The content of GABA in the diets was checked by the AccQ-Tag method (Pawlowska et al., 1993) with high-performance liquid chromatography (Alliance 2690 model, Waters Co., MA, USA). All were allowed the diet and tap water ad libitum and were housed in stainless-steel wire-bottomed cages under a 12-h light/dark cycle (lights on, 0830 to 2030).

Each rat was housed separately in a metabolic cage for 24 h to allow us to examine food and water consumption and to collect urine. The ambient temperature and humidity were controlled at 24±2 °C and 60±5%, respectively.

This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals, as adopted by the Committees for Animal Experiments of Yakult Central Institute.

### 2.2. Measurement of blood pressure and heart rate (Experiments 1 and 2)

After the rats had been kept at 37 °C in a constant temperature box for a few minutes, the systolic blood pressure and heart rate were measured between 0930 and 1030 every week by the tail-cuff method, using a programmed electrosphygmomanometer (Automatic Monitoring System UR-5000, Ueda-Seisakusho Co. Ltd., Tokyo, Japan). To avoid stress, the rats were habituated to the procedure by being handled by the experimenters for a few minutes on each of several days before the actual experiment.

### 2.3. Measurement of plasma renin activity and urinary sodium

Blood samples were taken from an indwelling fermoral artery cannula and then transferred into tubes containing EDTA-2Na (1 mg/ml) and immediately cooled. Plasma was promptly separated by centrifugation at 1500×*g* for 15 min at 4 °C and then stored at −80 °C until it was assayed. Plasma renin activity was measured by the radioimmunoassay method (DiaSorin Inc., Saluggi, Italy), following the manufacturer's instructions exactly. Values are expressed as nanograms of angiotensin I generated per millilitre of plasma per hour. Urine was collected into a container. At the end of each 24-h collection period the collection funnel was rinsed with 10 ml distilled water, and the wash was saved for determination of sodium content. The sodium and creatinine concentrations of the urine were measured by the ion electrode method and by enzyme assay, respectively (Automatic Clinical Analyzer 7170, Hitachi Co. Ltd., Tokyo, Japan).

### 2.4. Renal denervation

In Experiment 2, renal denervation was accomplished under ether anaesthesia through a ventral incision. This was done by stripping the renal adventitia and painting both of the renal arteries with 10% phenol in absolute ethanol (Huang et al., 1998). The sham operation consisted of a similar ventral incision and exposure of the renal arteries, but with no further intervention.

### 2.5. Additional analyses

Each kidney was examined macroscopically and weighed at 12 weeks of age.

### 2.6. Statistical analyses

Where appropriate, values are given as means±S.E.M. Changes from baseline values to post-administration values were analyzed by Dunnett's test. Two-way ANOVA was used for comparisons among the various groups (fed the GABA-free or the GABA-containing diet and renal-nerve-denervated or sham-operated). Where the variance ratio ($F$) was significant among groups, mean values of blood pressure or other parameters were compared by Tukey's test. All statistical analyses were two-tailed, and statistical significance was accepted at $P<0.05$. Data were analyzed by using the SAS System Ver. 6.12 (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. Effect of GABA in Experiment 1

The content of GABA in the GABA-containing diet was 0.5 g $kg^{-1}$, and no GABA was detected in the control (AIN-

Table 1
Experiment 1: Time-course data for food intake, water intake, body weight, urine volume, urinary Na, and Na/creatinine in spontaneously hypertensive rats treated with GABA ($n=10$ in each group)

| | Age (weeks) | Food intake (g 24 $h^{-1}$) | Water intake (ml 24 $h^{-1}$) | Body weight (g) | Urine volume (ml 24 $h^{-1}$) | Urinary Na (nmol 24 $h^{-1}$) | Na/creatinine (nmol $mg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Control | 8 | 18.2±0.5 | 25.4±1.2 | 190.6±1.3 | 10.1±0.6 | 2.6±0.1 | 0.65±0.01 |
| | 9 | 20.2±0.5 | 26.2±0.6 | 229.5±1.6 | 10.9±0.8 | 2.8±0.1 | 0.60±0.02 |
| | 10 | 19.8±0.5 | 24.4±0.8 | 263.2±2.3 | 11.5±0.8 | 2.8±0.1 | 0.57±0.02 |
| | 11 | 20.8±0.8 | 25.9±1.2 | 285.8±3.2 | 13.9±0.7 | 3.0±0.1 | 0.50±0.01 |
| | 12 | 18.9±0.3 | 25.1±0.9 | 305.9±3.2 | 15.1±0.7 | 2.9±0.1 | 0.43±0.01 |
| GABA | 8 | 18.6±0.7 | 24.4±1.0 | 190.6±1.4 | 10.3±0.8 | 2.6±0.1 | 0.63±0.02 |
| | 9 | 19.0±0.6 | 29.0±1.0* | 231.0±1.9 | 11.7±0.9 | 2.9±0.1 | 0.65±0.02 |
| | 10 | 18.8±0.4 | 28.1±1.4* | 259.8±2.5 | 12.4±1.3 | 3.1±0.2 | 0.64±0.02* |
| | 11 | 20.7±0.3 | 28.6±1.3 | 281.8±2.6 | 14.9±1.0 | 3.2±0.2 | 0.55±0.04 |
| | 12 | 18.6±0.3 | 27.7±0.9 | 300.8±2.9 | 14.9±1.0 | 3.2±0.2 | 0.53±0.02** |

Values are expressed as means±S.E.M. *$P<0.05$ vs. control at same time-point.

93G) diets. In Experiment 1, food consumption and body weight were not different between the GABA-fed and control groups at the same ages. The average values obtained for food consumption and body weight at 12 weeks of age were 18.6±0.3 g 24 $h^{-1}$ and 300.8±2.9 g in the GABA group, as against 18.9±0.3 g 24 $h^{-1}$ and 305.9±3.2 g in the control group. The average dose of GABA in the GABA-fed group was 27.6±0.4 mg $kg^{-1}$ 24 $h^{-1}$ during the experiment period; this was calculated from the amount of food consumed, the content of GABA in the diet, and the body weight. Water intake, however, was slightly but significantly higher in the GABA than in the control group ($P<0.05$ at both 9 and 10 weeks of age) (Table 1).

The time course of the changes in systolic blood pressure occurring during Experiment 1 is indicated in Fig. 1. In the control group, systolic blood pressure increased gradually with age, to exceed 220 mm Hg at 9 weeks of age. A significantly smaller increase in systolic blood pressure (versus the control group) was already evident at 1 week after the start of feeding with the GABA-containing diet ($P<0.05$), and a significant intergroup difference was maintained throughout the period on this diet. After 4 weeks of feeding on the test diets (i.e. at 12 weeks of age), the systolic blood pressure values in the control and GABA groups were 235.3±4.7 mm Hg and 216.9±2.6 mm Hg, respectively ($P<0.01$). There were no significant changes in heart rate in either the control or GABA group (data not shown).

The time courses of the changes in urine volume and urinary sodium excretion are shown in Table 1. These parameters tended to be slightly higher in the GABA group than in the control group, but there were no statistical differences between the two groups, except in the case of the urinary sodium to creatinine ratio at 10 and 12 weeks of age. Fig. 2 illustrates the effect of the GABA diet on plasma renin activity. A significantly lower level with respect to the control group was observed at 2 weeks after the start of feeding with the GABA diet (i.e. at 10 weeks of age) ($P<0.05$), and this difference tended to be present at the end of the period of feeding ($P=0.07$ at 12 weeks of age).

### 3.2. Effect of GABA in spontaneously hypertensive rats subjected to bilateral renal sympathetic denervation (Experiment 2)

There were no significant differences in the initial values obtained for body weight, systolic blood pressure, heart rate or plasma renin activity among the four groups of spontaneously hypertensive rats at 7 weeks of age (data not shown). The weekly systolic blood pressure values obtained for the four groups throughout the experimental period are shown in Fig. 3.

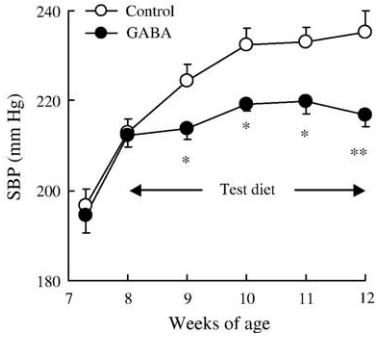


Fig. 1. Experiment 1. Time-course data for systolic blood pressure in spontaneously hypertensive rats treated with GABA. Each data-point represents the mean±S.E.M. for 10 animals. SBP, systolic blood pressure. *$P<0.05$ and **$P<0.01$ vs. control at same time-point.

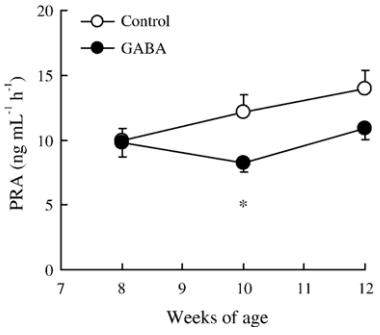


Fig. 2. Experiment 1. Time-course data for plasma renin activity in spontaneously hypertensive rats treated with GABA. PRA, plasma renin activity. Otherwise as in Fig. 3.

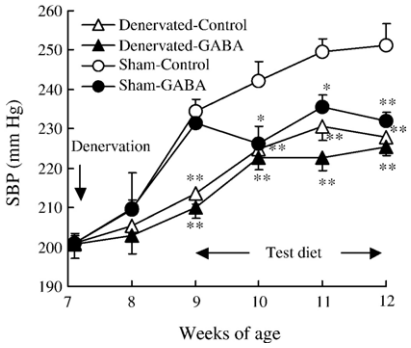


Fig. 3. Experiment 2. Effect of GABA administration on development of hypertension in bilaterally renal-denervated or sham-operated spontaneously hypertensive rats. Each data-point represents the mean±S.E.M. for 10 animals. SBP, systolic blood pressure. *$P<0.05$ and **$P<0.01$ vs. GABA-free, sham-operated group ("sham-control") at same time-point.

In the sham-operated control group, the systolic blood pressure increased gradually with age, from 201.1±1.4 mm Hg at 7 weeks to 251.3±5.5 mm Hg at 12 weeks of age. In contrast, renal-sympathetic-denervated animals showed a significantly slower development of hypertension. At the end of the first 2 weeks after surgery (i.e. at 9 weeks of age), the mean systolic blood pressure in the renal-sympathetic-denervated group was approximately 20 mm Hg lower than in the sham-operated control group. This difference was statistically significant ($P<0.01$) and was maintained until the end of the experiment. In the sham-operated GABA-fed group, however, systolic blood pressure did not increase significantly during the period on the test diet; it was approximately 15 mm Hg lower than that of the sham-operated control group throughout the GABA-intake period. In contrast, in the renal-denervated group, administration of GABA had no effect on systolic blood pressure.

A number of parameters at 12 weeks of age in Experiment 2 are shown in Table 2. The values obtained for body weight, food intake and water intake were not significantly different among the four groups. The kidney was significantly heavier in the renal-sympathetic-denervated group than in the sham-operated control group ($P<0.05$), with or without GABA treatment. The plasma renin activity was significantly higher in the sham-operated control group than in the other three groups. Food and water intake volumes per body weight did not change throughout the experimental period (age 7 to 12 weeks). There were no significant changes in heart rate in any group over this period (data not shown).

## 4. Discussion

The aim of our study was to confirm the hypotensive effect of chronically administered GABA and to clarify whether the renal nerves were involved in this phenomenon. Our findings indicate that, in spontaneously hypertensive rats, long-term oral administration of GABA leads to a decrease in blood pressure accompanied by a decrease in plasma renin activity. The mediation of this hypotensive effect appears to involve the renal nerves, to judge from the results in renal-denervated spontaneously hypertensive rats. Additionally, we measured the blood pressure at a consistent time of day in these experiments, but it was not taken into consideration in that time zone. Because the studies of long-term oral administration of GABA in humans have shown that the antihypertensive effect persists for a while after cessation of treatment (Inoue et al., 2003). Therefore, blood pressure was measured between 1 and 2 h after the lights were turned on, as described in Materials and Methods.

It has been reported that both central and systemic administration of GABA cause a reduction in blood pressure (Gillis et al., 1980; Defeudis et al., 1981; Defeudis, 1982). In a study in which GABA-rich tea was administered orally to rats (4 mg GABA per rat per day) for 4 weeks, blood pressure was significantly decreased, whereas the plasma GABA concentration was significantly higher than in rats receiving GABA-free water (Abe et al., 1995). In our experiment, the average GABA intake was 9 mg per rat per day (calculated from the amount of food consumed). Thus, a hypotensive effect can be achieved by chronic oral administration of these amounts of GABA.

The mechanism underlying the hypotensive action of systemically administered GABA has not been fully elucidated, although several hypotheses have been proposed. GABA crosses the blood–brain barrier poorly owing to its low lipid solubility (Kuriyama and Sze, 1971; Haywood et al., 1985) and its concentration in the brain does not change following an i.p. or i.v. injection (Gelder and Elliot, 1958). GABA has been shown to inhibit noradrenaline release from sympathetic nerve terminals within the mesenteric arterial bed of spontaneously hypertensive rats (Hayakawa et al., 2002), and this effect may

Table 2
Experiment 2: Body weight, food intake, water intake, kidney weight and plasma renin activity (PRA) measured in spontaneously hypertensive rats ($n=10$ in each group) at age 12 weeks (at the end of the experiment)

| | Body weight (g) | Food intake (g 24 $h^{-1}$) | Water intake (g 24 $h^{-1}$) | Kidney weight (g) | PRA (ng $ml^{-1}$ $h^{-1}$) |
|---|---|---|---|---|---|
| Control | | | | | |
| Denervated | 300.3±2.4 | 16.5±0.5 | 32.8±0.9 | 2.41±0.06[a] | 9.5±7.2[a] |
| Sham-operated | 299.4±3.0 | 16.4±0.4 | 29.8±1.4 | 2.32±0.03[b] | 15.5±8.8[b] |
| GABA | | | | | |
| Denervated | 306.9±3.0 | 16.9±0.4 | 32.8±0.9 | 2.46±0.04[a] | 9.5±3.3[a] |
| Sham-operated | 302.4±3.6 | 16.7±0.4 | 33.6±1.5 | 2.30±0.02[b] | 9.5±3.6[a] |

Values are expressed as means±S.E.M. Data with different superscript letters are significantly different from each other ($P<0.05$).

be mediated through peripheral activation of $GABA_B$ receptors, because the resulting hypotensive effect is reduced by pre-administration of the $GABA_B$-receptor antagonist saclofen (Kimura et al., 2002). Although this action of GABA may well be involved in its hypotensive effect, its involvement may be transient. Certainly, we cannot presume that it explains the long-term hypotensive effect induced by chronic administration of GABA.

The kidney plays an important role in the long-term control of blood pressure through its regulation of the electrolyte composition of body fluids and its release of various vasoactive substances, such as renin–angiotensin (Guyton et al., 1972; Hall, 2003). In fact, blood pressure in spontaneously hypertensive rats can be normalized by means of a kidney graft from a normotensive Wistar–Kyoto rats (Grisk et al., 2002), and renal transplantation has long-term effects on the recipient's plasma renin activity (Rettig et al., 1994) and cumulative sodium retention (Graf et al., 1993). It is well known that kidney function is strongly affected via the sympathetic innervation (Winternitz et al., 1980; Mark, 1996). Moreover, renal sympathetic nerve activity is markedly greater in spontaneously hypertensive rats than in age-matched normotensive Wistar–Kyoto rats (Lundin et al., 1984). In previous studies of the effects of GABA on renal function: (a) in the isolated perfused rat kidney, GABA agonists increased perfusion pressure and diminished the glomerular filtration rate, with accompanying increments in the fractional excretions of water and sodium (Monasterolo et al., 1996), and (b) in the isolated rat kidney, GABA suppressed renal sympathetic neurotransmitter release via presynaptic $GABA_B$ receptors, thereby attenuating noradrenaline-mediated vasoconstriction (Fujimura et al., 1999).

By performing bilateral renal sympathetic denervation in spontaneously hypertensive rats, we tried to clarify whether modulation of the effects of renal sympathetic nerve activity is involved in the expression of the hypotensive effect of GABA. As mentioned above, in our spontaneously hypertensive rats the natriuresis (which is important for the regulation of blood pressure) was only slightly increased by GABA administration (and only when measured as sodium per creatinine). Other investigators who have examined natriuresis in renal-denervated spontaneously hypertensive rats have obtained conflicting results. After renal denervation, urinary sodium excretion and urine flow have been found to increase in spontaneously hypertensive rats (Liard, 1997) but not in normotensive Wistar–Kyoto rats (Kubota et al., 1993). By contrast, other investigators have found that after renal denervation in spontaneously hypertensive rats natriuresis shows almost no change (Greenberg and Osborn, 1994). Because natriuresis has a lowering effect on blood pressure, the weak natriuretic effect of GABA seen in our study may contribute only slightly to the observed hypotensive effect of GABA. The ratio of urinary sodium to creatinine decreased with age in both the GABA-fed group and the control group (Table 1). The same phenomenon has been reported before and was assumed to reflect an increase in the muscle mass of these growing male rats (Takishita et al., 1996). The kidneys were significantly heavier in both renal-denervated groups. This phenomenon also has been reported before, but dry weight of kidney was not different between in the sham operation and in the denervation (Tomoda et al., 1997). It may be increase in retention of water in the kidney, but we do not know the reason behind this hypertrophy.

As mentioned above, renal sympathetic nerve activity promotes anti-natriuresis and the release of renin, in addition to its effect of increasing renal vascular resistance (Dibona, 1989). It is well known that after renal denervation in spontaneously hypertensive rats the levels of both renal noradrenaline and plasma renin activity are significantly lower than those seen in intact animals (Kubota et al., 1993; Kassab et al., 1995). We found here that GABA had much the same effects as renal denervation on blood pressure and plasma renin activity. In fact, we found that plasma renin activity was significantly lower (at least at one time point) in the GABA-fed group in Experiment 1 than in the control, and in both GABA groups in Experiment 2 than in the sham-operated control. Similarly, plasma renin activity was significantly lower in the renal-sympathetic-denervated group in Experiment 2 than in the sham-operated controls. Regardless of these findings, GABA had no effect on either blood pressure or plasma renin activity in the renal-sympathetic-denervated spontaneously hypertensive rats. These results suggest that GABA may reduce renin release from the kidney by reducing the effects induced by the renal sympathetic nerves. If this is indeed the case, the following sequence of events would be predicted to underlie the GABA-induced long-term hypotensive effect seen in spontaneously hypertensive rats: (i) GABA acts on $GABA_B$ receptors on the sympathetic nerve endings in the kidney to inhibit the release of noradrenaline; (ii) renin release is reduced; (iii) angiotensin II formation is reduced owing to the decreased plasma concentration of renin; and thereby (iv) a long-term hypotensive effect results.

In conclusion, we have shown that long-term administration of GABA significantly decreases blood pressure and plasma renin activity in sham-operated spontaneously hypertensive rats but not in renal-sympathetic-denervated spontaneously hypertensive rats. This result suggests that the GABA-induced hypotensive effect may be mediated by a reduction in the effects of renal sympathetic nerve activity in spontaneously hypertensive rats.

## Acknowledgments

We thank Mr. S. Thuchikura (Disease Model Cooperative Research Association, Kyoto, Japan) for his skilled technical assistance. We are also grateful to Professor K. Kamata (Hoshi University, Tokyo, Japan) for critically reading the manuscript.

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