Involvement of γ -Aminobutyric Acid (GABA) B Receptors in the Hypotensive Effect of Systemically Administered GABA in Spontaneously Hypertensive Rats

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ABSTRACT—We investigated the effects of intraduodenally (i.d.) administered γ -aminobutyric acid (GABA) on blood pressure (BP) in anesthetized spontaneously hypertensive rats (SHR) and the mechanism underlying this effect, especially the type of GABA receptor involved in the depressive effect of this amino acid. GABA (0.3 to 300 mg/kg, i.d.) caused a dose-related decrease in the BP of 9.20 ± 3.96 to 35.0 ± 5.34 mmHg (mean $\pm$ S.E.M.) that lasted for 30 to 50 min. The minimum effective i.d. dose of GABA was 0.3 to 1.0 mg/kg. Results pertaining to the mechanism underlying the GABA-induced effects on BP were as follows: a) GABA did not alter the BP-related effects of exogenous noradrenaline and acetylcholine; b) pretreatment with hexamethonium decreased the GABA-induced fall in BP, and GABA tended to reduce the pressor response associated with injection of dimethyl phenylpiperazinium; and c) pretreatment with 2-hydroxysaclofen markedly reduced the GABA-induced drop in BP, whereas pretreatment with bicuculline did not. In conclusion, in SHR, low-dose (0.3 to 1.0 mg/kg, i.d.) GABA had a hypotensive effect, which may result from attenuation of sympathetic transmission through the activation of GABA_B receptors at presynaptic or ganglionic sites.

Keywords: γ -Aminobutyric acid (GABA), Blood pressure, Spontaneously hypertensive rat, GABA receptor, Sympathetic nerve

It is well known that γ -aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system (CNS) (1), and the central administration of GABA or GABA agonist decreases blood pressure (BP) by reducing sympathetic tone (2–4) or by disrupting the angiotensin II (5, 6). Thus it has been confirmed that GABA plays an important role in CNS control of BP.

Oral administration of GABA (3 g daily) to hypertensive patients was first reported about four decades ago, and it was found that oral GABA decreased their BPs without affecting their heart rates (7). Considering that a high dose of GABA administered systemically can reach the brain (8), this effect is thought to occur via a central mechanism. However, a controlled trial of oral GABA had not been conducted until recently, because GABA is thought to cross the blood-brain barrier only minimally (9–11). Therefore most efforts have been concentrated on designing highly hydrophobic derivatives of GABA that can easily permeate brain tissue. Recently, several studies in Japan have

addressed the hypotensive effects of various GABA-containing dietary supplements and other products (12–14). These efforts showed that the chronic ingestion of a low dose of GABA decreased systolic BP in spontaneously hypertensive rats (SHR). Because low-dose GABA is not thought to cross the blood-brain barrier, the agent's hypotensive effect has been attributed to a peripheral mechanism.

Several mechanisms (including ganglionic blockade, activation of GABAergic receptors, direct action on vasculature, and inhibition of transmitter release from sympathetic nerve terminals) have been postulated to underlie the hypotensive action of GABA in the peripheral vasculature but are not completely understood (15–18). Furthermore, results from in vivo studies apparently contradict those from in vitro work regarding the type of GABA receptor involved. Namely, GABA_A receptor antagonists (e.g., picrotoxin, bicuculline) reduced the hypotensive action of intravenous GABA (16, 19–21), whereas the GABA_B antagonist 2-hydroxysaclofen (but not a GABA_A-receptor antagonist) counteracted the vasodilatory action of GABA in experiments using isolated pulmonary (22), renal (23),

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or mesenteric (24) arteries. Therefore, we designed our investigation first to clarify the minimum effective intraduodenal dose of GABA to confirm whether a low dose of oral GABA has real potential to cause a hypotensive effect. Second, we sought to elucidate the type of receptor involved in the peripheral mechanism underlying the hypotensive action of GABA.

MATERIALS AND METHODS

Animals and measurements

Male SHR/Izm (280 to 330 g) were purchased from Tokyo Experimental Animals (Tokyo), housed 3 per cage in a room maintained at $22 \pm 2^\circ\text{C}$ and at a relative humidity of $55\% \pm 20\%$, and allowed to acclimate for at least 1 week before entering the study. The rats were anesthetized by intraperitoneal (i.p.) injection of a mixture of urethane (500 mg/kg; Sigma, St. Louis, MO, USA) and α -chloralose (50 mg/kg; Sigma).

To measure BP, the left femoral artery was cannulated, and the cannula was connected to a forced pressure transducer (Blood pressure monitoring kit; Nihon Kohden, Tokyo) and amplifier (AP-621G, Nihon Kohden). Heart rate was recorded with a heart rate meter (AT-601G, Nihon Kohden) and amplifier, which were triggered by the systolic blood pressure.

Drugs

GABA was purchased from Wako Pure Chemicals (Tokyo), *dl*-noradrenaline (Nad) from Sankyo (Tokyo), acetylcholine (ACh) from Daiichi Pharmaceutical (Tokyo), bicuculline and 2-hydroxysaclofen from Tocris Cookson (Ellisville, MO, USA), and other chemicals from Sigma.

For i.d. administration, GABA was dissolved in distilled water; GABA for i.v. administration and other chemicals were dissolved in saline. The volumes given were 0.05 to 0.1 mL/100 g body weight for i.v. administration and 0.5 mL/100 g for i.d. and i.p. administration.

Administration

The right femoral vein was cannulated for i.v. drug administration. The stomach wall was partly dissected, and the duodenum was cannulated through the stomach for i.d. administration.

When the time interval between injections of GABA was short, the BP depression was attenuated, similar to the response seen during tachyphylaxis. Therefore, for experiments requiring repeated injection of GABA, it was administered every 20 min for i.v. administration and at >40-min intervals for i.d. administration.

The data available in the literature are unclear regarding the initiation and duration of the effect of the various GABA-receptor antagonists we studied. Therefore, we per-

formed separate time-course experiments and monitored the effects on BP until 45 min after the administration of the particular antagonist. We then injected challenge doses of GABA during the interval appropriate to the agonist (as defined through the time-course experiment) and compared the magnitude of the BP depression obtained after administration of both antagonist and GABA with that generated by injecting GABA only.

Data reporting and statistical analyses

The BP before the administration of drugs (baseline BP) was obtained by calculating the average blood pressure (ABP): $\text{ABP} = [\text{systolic BP} + \text{diastolic BP}] / 2$. After the administration of drugs, BP was reported as the ABP after administration of i.d. GABA, as the systolic BP (SBP) to monitor the maximal pressor response to Nad or dimethyl phenylpiperazinium (DMPP), and as the diastolic BP (DBP) to follow the maximal depressor response to ACh or i.v. GABA. The relative change (%) was calculated as $(\text{absolute change}) / \text{baseline BP} \times 100\%$.

The data were analyzed by using Student's unpaired *t*-test (SAS system ver.6.12; SAS Institute, Inc., Cary, NC, USA), and differences were considered to be significant at $P < 0.05$. All values are presented as the mean $\pm$ S.E.M.

RESULTS

Dose-related hypotension after intraduodenal administration of GABA

The dose-response curve of the depressor effect of i.d. GABA and a representative chart are shown in Figs. 1 and 2, respectively. The onset times of the depressor effect (4 to 6 min) were almost the same among doses from 10 to 300 mg/kg. However, the onset of the depressor effect apparently was delayed (17 to 24 min) at doses less than 3 mg/kg. Dose-related decreases in BP occurred between 0.3 and 300 mg/kg; the baseline BP was 148 mmHg, the absolute decrease was 10 to 35 mmHg, and the relative fall ranged from 7% to 25%. In a typical pattern of hypotension in response to i.d. GABA (Fig. 2), the BP gradually decreased over 30 to 40 min, and the heart rate was not affected (data not shown).

In contrast, i.v. injection of GABA immediately reduced the BP, which then recovered gradually (Fig. 3). The maximum depression in BP after i.v. administration was much greater than that after i.d. administration. The duration rather than the magnitude of depression tended to be increased as doses of GABA increased.

Effect of GABA on the pressor response to Nad and the depressor response to ACh

We compared the pressor response to Nad (5 $\mu\text{g/kg}$, i.v.) and the depressor response to ACh (2 $\mu\text{g/kg}$, i.v.) after

Fig. 1. Dose-response curve of maximum hypotension due to administration of intraduodenal GABA to spontaneously hypertensive rats. The values show the absolute (mmHg) decrease in the average blood pressure. Each point represents the mean $\pm$ S.E.M. of 6 to 13 rats, except for the 10-mg/kg group ($n = 2$).

administration of GABA (3 or 5 mg/kg, i.v.) with those in the absence of GABA. Administration of GABA had little effect on these responses (Tables 1 and 2). The same result was obtained for i.v. injection of GABA (data not shown).

Effects of ganglionic blocking agents and stimulants

The depression of the GABA-associated decrease in BP and its duration were attenuated significantly (BP, $P < 0.05$; duration, $P < 0.01$) by treatment with hexamethonium (3 mg/kg, i.v.; Table 3). After we confirmed the pressor response (average, 80 mmHg) to DMPP (10 to 100 μ g/kg, i.v.), we

Fig. 3. Pattern of hypotension after intravenous injection of GABA (0.3, 1, 3 or 5 mg/kg) in spontaneously hypertensive rats anesthetized with urethane (500 mg/kg) and α -chloralose (50 mg/kg). Arrowhead, GABA administration.

then administered GABA (100 or 300 mg/kg, i.d.), followed by DMPP injection. At 300 mg/kg GABA, the pressor response to DMPP tended to be reduced by an average of 51 mmHg (40%; $n = 6$; $P = 0.053$); however, no change was observed in the relative rise in BP at 100 mg/kg

Fig. 2. Pattern of hypotension after intraduodenal administration of GABA (0.3, 1 or 3 mg/kg) in spontaneously hypertensive rats anesthetized with urethane (500 mg/kg) and α -chloralose (50 mg/kg). Arrowhead, GABA administration.

Table 1. Effect of GABA on the noradrenaline (Nad)-induced increase of blood pressure (BP) in spontaneously hypertensive rats

Dose of GABA (mg/kg)	Before GABA			After GABA		
	Baseline ^a (mmHg)	Nad		Baseline ^a (mmHg)	Nad	
		Absolute change ^b (mmHg)	Relative rise ^c (%)		Absolute change ^b (mmHg)	Relative rise ^c (%)
3	117 ± 8.33	87.5 ± 9.47	77.0 ± 10.0	118 ± 19.6	82.5 ± 11.3	76.1 ± 19.0
5	135 ± 6.30	43.3 ± 5.47	33.8 ± 5.53	118 ± 12.0	61.7 ± 10.1	52.8 ± 15.8

Noradrenaline (5 µg/kg, i.v.) was administered before and after intravenous administration of GABA. Values are the mean ± S.E.M. of the data from 3 rats. ^a(Systolic blood pressure + diastolic blood pressure) / 2. ^bThe difference between the baseline and maximum systolic blood pressures. ^cAbsolute change / baseline BP × 100%.

Table 2. Effect of GABA on the acetylcholine (ACh)-induced decrease of blood pressure in spontaneously hypertensive rats

Dose of GABA (mg/kg)	Before GABA			After GABA		
	Baseline ^a (mmHg)	ACh		Baseline ^a (mmHg)	ACh	
		Absolute change ^b (mmHg)	Relative fall ^c (%)		Absolute change ^b (mmHg)	Relative fall ^c (%)
3	116 ± 6.82	-45.8 ± 8.21	38.5 ± 4.94	135 ± 11.5	-53.3 ± 8.82	39.0 ± 3.16
5	145 ± 7.64	-67.5 ± 6.29	46.2 ± 2.25	136 ± 9.61	-60.0 ± 10.0	44.8 ± 1.95

Acetylcholine (2 µg/kg, i.v.) was administered before and after intravenous administration of GABA. Values are the mean ± S.E.M. of the data from 3 rats. ^a(Systolic blood pressure + diastolic blood pressure) / 2. ^bThe difference between the baseline and minimum diastolic blood pressures. ^cAbsolute change / baseline × 100%.

Table 3. Effect of hexamethonium (Hex) on the GABA-induced decrease of blood pressure in spontaneously hypertensive rats

Dose of GABA (mg/kg)	Before Hex				After Hex			
	Baseline ^a (mmHg)	GABA		Duration of hypotension (min)	Baseline ^a (mmHg)	GABA		Duration of hypotension (min)
		Absolute change ^b (mmHg)	Relative fall ^c (%)			Absolute change ^b (mmHg)	Relative fall ^c (%)	
3	157 ± 10.9	-68.3 ± 5.27	45.0 ± 3.35	8.20 ± 1.15	98.8 ± 19.4	-15.0 ± 5.40 ^d	15.2 ± 3.62 ^e	2.10 ± 1.96 ^f
5	150 ± 7.64	-61.7 ± 3.33	40.1 ± 2.16	12.5 ± 2.50	103 ± 28.9	-13.3 ± 10.9 ^f	9.20 ± 6.85 ^f	0.10 ± 0.05 ^f

GABA was administered intravenously before and after administration of hexamethonium (3 mg/kg, i.v.). Values are the mean ± S.E.M. of the data from 4 rats. ^a(Systolic blood pressure + diastolic blood pressure) / 2. ^bThe difference between the baseline and minimum diastolic blood pressures. ^cAbsolute change / baseline × 100%. ^d*P* < 0.001 (Student's unpaired *t*-test) versus before injecting Hex. ^e*P* < 0.01 (Student's unpaired *t*-test) versus before injecting Hex. ^f*P* < 0.05 (Student's unpaired *t*-test) versus before injecting Hex.

GABA (Table 4).

Effect of GABA receptor antagonists

Table 5 shows the GABA (0.3 mg/kg, i.v.)-associated BP depression before and after the administration of bicuculline (1 mg/kg, i.v.), a GABA_A-receptor antagonist. The BP was slightly increased after the administration of bicuculline (data not shown), but the magnitude of the GABA-associated BP depression (27.2 mmHg, 22.4%) before the administration of the antagonist was equivalent to that induced by GABA at 3 min after injection of bicuculline (28.8 mmHg, 22.7%) and at 25 min after bicuculline administration (31.7 mmHg, 23.9%). The duration of the response was also unaffected.

The BP depression due to GABA (0.3 mg/kg, i.v.) before and after the administration of the GABA_B antagonist 2-hydroxysaclofen (1 mg/kg, i.v.) is shown in Table 6. After the administration of 2-hydroxysaclofen, the BP was slightly increased, as for bicuculline (data not shown). Both the magnitude and duration of the depressor response to GABA were reduced at 3 and 25 min after the administration of 2-hydroxysaclofen. At 45 min after injection of the antagonist, the reduction had dissipated.

DISCUSSION

Our present study confirms that a low dose of i.d. GABA decreases the BP of SHR. We observed a linear dose-

Table 4. Effect of GABA on the dimethyl phenylpiperazinium (DMPP)-induced increase in the blood pressure (BP) of spontaneously hypertensive rats

Dose of GABA (mg/kg)	Before GABA			After GABA		
	Baseline ^a (mmHg)	DMPP		Baseline ^a (mmHg)	DMPP	
		Absolute change ^b (mmHg)	Relative rise ^c (%)		Absolute change ^b (mmHg)	Relative rise ^c (%)
100	145 ± 13.2	93.3 ± 6.67	64.7 ± 2.03	124 ± 13.1	75.0 ± 2.89	61.2 ± 6.56
300	129 ± 5.39	80.0 ± 11.4	61.9 ± 10.2	123 ± 5.58	50.8 ± 6.88	39.8 ± 5.74

DMPP (10 to 100 µg/kg, i.v.) was administered before and after intraduodenal administration of GABA. Values are the mean ± S.E.M. of the data from 3 to 6 rats. ^a(Systolic blood pressure + diastolic blood pressure) / 2. ^bThe difference between the baseline and maximum systolic blood pressures. ^cAbsolute change / baseline × 100%.

Table 5. Effect of bicuculline (Bic) on the GABA-induced decrease in the average blood pressure^a of spontaneously hypertensive rats

	Before Bic	Min after Bic administration	
		3	25
Baseline (mmHg) ^a	121 ± 4.00	126 ± 5.07	131 ± 5.43
GABA			
Absolute change (mmHg) ^b	27.2 ± 0.88	28.8 ± 1.83	31.7 ± 3.58
Relative fall (%) ^c	22.4 ± 0.39	22.7 ± 0.86	23.9 ± 2.10
Duration (min)	5.10 ± 0.50	5.50 ± 0.64	6.00 ± 1.34

GABA (0.3 mg/kg, i.v.) was administered before and at various points after administration of Bic (1 mg/kg, i.v.). Values are the mean ± S.E.M. of the data from 5 to 9 rats. ^a(systolic blood pressure + diastolic blood pressure) / 2. ^bThe difference between the baseline and minimum diastolic blood pressures. ^cAbsolute change / baseline × 100%.

Table 6. Effect of the receptor 2-hydroxysaclofen (2-Hy) on the GABA-induced decrease in the average blood pressure^a of spontaneously hypertensive rats

	Before 2-Hy	Min after 2-Hy administration		
		3	25	45
Baseline (mmHg) ^a	127 ± 4.22	128 ± 4.53	129 ± 3.98	127 ± 3.74
GABA				
Absolute change (mmHg) ^b	27.5 ± 2.14	20.6 ± 2.58	20.6 ± 1.48 ^d	25.0 ± 2.24
Relative fall (%) ^c	21.7 ± 1.41	16.1 ± 1.94	16.1 ± 1.25 ^d	19.7 ± 1.84
Duration (min)	4.7 ± 0.57	2.70 ± 0.53 ^d	2.80 ± 0.24 ^d	2.80 ± 0.82

GABA (0.3 mg/kg, i.v.) was administered before and at various points after the administration of 2-Hy (1 mg/kg, i.v.). Values are the mean ± S.E.M. of the data from 5 to 9 rats. ^a(systolic blood pressure + diastolic blood pressure) / 2. ^bThe difference between the baseline and minimum diastolic blood pressures. ^cAbsolute change / baseline × 100%.

^d*P* < 0.05 (Student's unpaired *t*-test) versus before 2-Hy administration.

related response for i.d. GABA doses of 0.3 to 300 mg/kg body weight. Although the BP depression for the group receiving 0.3 mg/kg, i.d. was 9.2 ± 4.0 mmHg, 2 of the 6 rats in that group showed no decrease. This result suggests that the minimum effective i.d. GABA dose is actually 0.3 to 1.0 mg/kg. In recent studies of various GABA-containing dietary supplements and other products, regular ingestion of GABA at approximately 1 mg/kg daily for a several weeks yielded a hypotensive effect in SHR (12, 14). The

effective dose of GABA we calculated is similar to that in these studies. Therefore, we have confirmed that low-dose oral GABA decreases the BP of hypertensive rats. Furthermore, we found that mildly hypertensive human patients who daily consumed a fermented milk product containing 10 mg GABA reduced their BPs after 4 weeks without affecting their heart rates (25).

Low-dose GABA is not thought to cross the blood-brain barrier (9–11); therefore, the result mentioned previously

can be attributed to a peripheral mechanism. Several groups have studied the effect of systemically administered GABA on the cardiovascular system of anesthetized normotensive animals. The results of these studies suggest that dose-related hypotensive effects occurred at doses of 1 to 1000 $\mu\text{g}/\text{kg}$ (15–17, 19). Several mechanisms underlying the hypotensive action of GABA in the peripheral vasculature have been postulated, including ganglionic blockade (16), activation of GABAergic receptors (16, 17), direct action on vasculature (15, 19), and inhibition of transmitter release from sympathetic nerve terminals (26, 27).

Administration of GABA to SHR affected neither the pressor response to Nad nor the depressor response to ACh. These findings suggest that GABA lacked a direct effect on Nad and ACh receptors, which occur on the smooth-muscle and endothelial cells of peripheral blood vessels, respectively.

We then evaluated the effect of the ganglionic stimulant DMPP and the ganglionic blocker hexamethonium on the GABA-induced hypotension of SHR. Because DMPP injection after i.v. administration of GABA failed to give a consistent response, we administered GABA intraduodenally, which caused a BP decrease of moderate magnitude and duration. In many animals, administration of GABA tended to reduce the transient pressor response to DMPP ($P = 0.053$). In addition, injection of DMPP prior to administration of GABA often inhibited the GABA-associated depression of the BP (data not shown). Blocking the ganglion by pretreatment with hexamethonium reduced the GABA-induced depression. Thus, GABA seems to inhibit sympathetic neurotransmission at sympathetic ganglia. However, the same effect probably would result if GABA inhibited sympathetic neurotransmission at the presynaptic sites of sympathetic nervous terminals. Regardless, GABA seems to have an effect on sympathetic neurons.

We assessed the effects of two types of GABA-receptor antagonists, bicuculline that acts on GABA_A receptors and 2-hydroxysaclofen that blocks GABA_B receptors, on the GABA-induced BP depression. Bicuculline did not affect the depression, which was apparently inhibited by 2-hydroxysaclofen. The effect of this inhibitory pattern was to shorten the duration of the depression by approximately one half rather than to attenuate the magnitude of the pronounced depression that occurs soon after i.v. administration of GABA.

Regarding the type of GABA receptor involved in this mechanism, *in vivo* studies have yielded results that are apparently contradictory to those from *in vitro* experiments. Administration of a GABA_A-receptor antagonist (picrotoxin, bicuculline) followed by administration of intravenous GABA in experimental animals reduced the hypotensive action of GABA (16, 19–21). In contrast, the GABA_B antagonist 2-hydroxysaclofen (but not GABA_A

antagonists) reduced the GABA-induced vasodilation in various excised peripheral artery models (22–24), and our findings agree with these *in vitro* results. Thus, primarily GABA_B receptors seem to be involved in the hypotensive effects due to low-dose GABA in SHR.

The reasons why GABA_A antagonist had no effect on the hypotensive action of GABA in our experiments may be explained as follows. In light of results from various *in vitro* studies, sympathetic nerve endings probably lack GABA_A receptors because GABA_A antagonists have no effect on this region (22–24). In contrast, ganglionic sites may contain only GABA_A receptors or both GABA_A and GABA_B receptors. Regardless of which scenario is correct, it must be accepted that GABA_A receptors occur at ganglionic sites but not sympathetic nerve endings. Considering the fact that the density of sympathetic innervation is greater in SHR than in normotensive age-matched Wistar Kyoto rats (28, 29), the hypotensive effect of systemically administered GABA is effectively concentrated at these nerve ending sites rather than at ganglionic sites in SHR. Therefore, the magnitude of the effect of blocking the GABA_A receptors at a ganglion will be less apparent in SHR than in normotensive animals. Conversely, in a hypertensive situation, GABA exerts a prominent effect in SHR because of the activation of the GABA_B receptors at sympathetic nerve endings.

GABA receptors are widespread throughout the peripheral, including in cells of the autonomic nervous system, endocrine cells, exocrine cells, and smooth muscle cells (30). Although there have been no definitive reports to date on the presence of GABA receptors in the peripheral blood vessels themselves, the catabolic enzyme of GABA, glutamate decarboxylase (31), and the metabolic enzyme GABA transaminase (32) occur in various peripheral vessels. More recently, Castelli et al. (33) found that the mRNAs of 2 isoforms of the GABA_B receptor occurred in all 12 organs examined, including the heart, lung, liver, and small and large intestines. In light of this result, it is reasonable to think that GABA receptors exist in the peripheral vessels of organs that play an important role in defining the systemic blood pressure. However, it remains necessary to demonstrate directly the presence of GABA receptors in these blood vessels.

In conclusion, a low dose of i.d. GABA decreases the BP of SHR; the minimum effective i.d. dose is 0.3 to 1.0 mg/kg. The mechanism underlying the GABA-associated depression of BP is not related to a direct effect of the drug on the blood vessels. Instead, this effect may be at least partly caused by attenuation of sympathetic neurotransmission following activation of GABA_B receptors at nerve ending presynaptic or ganglionic sites.

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