Investigations into the Mechanism of the Antihypertensive Effect of SGB-1534, a Novel α_1 -Adrenoceptor Antagonist, in Rats

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Abstract—Experiments in vitro and in vivo were undertaken to examine possible involvement of a central effect in the hypotensive mechanism of SGB-1534. SGB-1534 selectively antagonized the contraction of isolated rat aortae to phenylephrine with a pA_2 value of 10.57, 3.9 times higher than prazosin, and markedly displaced the α_1 -adrenoceptor ligand 3H -prazosin (pK_i : 8.81) in rat brain. In anesthetized spontaneously hypertensive rats (SHRs), SGB-1534 (0.3–3 μ g/kg) and prazosin (3–30 μ g/kg) given intravenously (i.v.) and intracerebroventricularly (i.c.v.) produced a dose-dependent and long-lasting depressor response associated with no change in heart rate (HR). The two drugs (i.c.v.), however, significantly attenuated the pressor response to i.v. noradrenaline. Single i.v. injections of SGB-1534, prazosin and yohimbine dose-dependently inhibited the St 587 (a highly specific and centrally acting α_1 -adrenoceptor agonist) enhanced flexor reflex and the pressor response to i.v. phenylephrine in pithed rats. However, the activities of SGB-1534 and prazosin in inhibiting the St 587-enhanced flexor reflex were 16,000 and 660 times, respectively, less than those in attenuating the pressor response to i.v. phenylephrine. It seems that the hypotensive action of SGB-1534 is due to the peripheral α_1 -adrenoceptor antagonistic mechanism rather than the central one.

Although the etiology of essential hypertension still remains unknown, there is no doubt that the sympathetic nervous system plays a major role in the pathogenesis and maintenance of essential hypertension (1–3). Recent pharmacologic advances have resulted in the discovery of drugs of proven clinical utility, which effectively reduce sympathetic input to the cardiovascular system at any possible site (either central or peripheral), leading to the reduction of blood pressure in a substantial portion of patients with essential hypertension (1, 4). SGB-1534 is a novel antihypertensive drug (5) currently undergoing clinical evaluation. A series of these experiments have revealed that the mechanism by which SGB-1534

lowers blood pressure is greatly dependent on interruption of the sympathetic nervous system via peripheral α_1 -adrenoceptor blockade (6–9). However, there are no precise investigations pertaining to the participation of any central component in the hypotensive effect of SGB-1534.

This report describes the antihypertensive activity of SGB-1534 in SHRs and discusses information relevant to the mechanism by which it lowers blood pressure. SGB-1534 was compared with prazosin, a highly selective α_1 -adrenoceptor antagonist (10), and yohimbine, a relatively selective α_2 -adrenoceptor antagonist (11) and clonidine.

Materials and Methods

In vitro studies

Isolated blood vessels: Male Wistar-Imamichi strain rats (about 350 g) were killed by decapitation, and then the thoracic aorta was removed and dissected free from

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connective tissue. The ring segments (2–3 mm length) were suspended under a load of 2 g in a 10 ml organ bath containing a modified Krebs-Henseleit bicarbonate solution of the following composition: 125.0 mM NaCl, 5.6 mM KCl, 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 2.5 mM CaCl_2 , 25.0 mM NaHCO_3 , 9.0 mM glucose and 0.06 mM L-ascorbic acid. The bath was aerated thoroughly with a gas phase mixture of 95% O_2 –5% CO_2 . The pH of the gassed solution was 7.4 at 37°C. The muscle segments were connected to a Nihon Kohden force transducer (SB-IT) for continuous recording of isometric tension. Recordings were made on a Yokogawa ink-writing pen recorder (model 3066). Five preparations were run concurrently. The muscle segments were equilibrated for at least 3 hr, with washes every 30 min, before exposure to drugs. The antagonist and agonist were added to the organ bath in a volume of 0.1 ml. Subsequent doses of agonists were increased by a factor of about 3 and were introduced when the response of the preceding ones had reached a steady level. Thus, a cumulative concentration-percentage maximal response curve to an agonist drug was constructed for each tissue. In the preliminary experiment, the curve for each agonist was constructed 3 times in the absence of any agonists. It was confirmed that there were no significant differences between the corresponding values obtained from the second and third curves. Therefore, after the second concentration-response curve was constructed, the tissue was washed out repeatedly in the absence of antagonists and equilibrated for at least 1 hr. An antagonist was added to the organ bath, and 10 min later, the cumulative concentration-response curve to an agonist drug was constructed again for each tissue. All drug concentrations are expressed as final molar (M) concentrations in the bath solution. The negative logarithm of molar concentration causing a 2-fold shift to the right of the concentration-response curve for the agonist (pA_2) was determined according to a Schild analysis (12).

Radioligand binding assay: Male Wistar-Imamichi strain rats (350–400 g) were used. The isolation of membrane fraction from rat

brain was carried out as reported by Nagatomo et al. (13). Briefly, after decapitation, the whole brain was quickly removed and minced with small scissors in 10 volumes of ice-cold 0.25 M sucrose and 5 mM Tris-HCl buffer (pH 7.5). The suspension was homogenized in a glass homogenizer. The homogenate was filtered through 4 layers of gauze, and the filtrate was centrifuged at $40,000\times g$ for 30 min at 4°C. The pellet obtained was resuspended in an incubation buffer containing 100 mM Tris-HCl buffer (pH 7.5) and 20 mM MgCl_2 . Protein concentration, as determined according to the method of Lowry et al. (14), was adjusted to 0.5 mg/ml for the ^3H -prazosin and for the ^3H -aminoclonidine binding assays.

Binding studies were performed by the displacement method. As radioligands, ^3H -prazosin (30 Ci/mmol) and ^3H -p-aminoclonidine (50 Ci/mmol) in ethanol were purchased from the New England Nuclear Co., Ltd., and stored at -20°C until use. In the case of the α_1 -adrenoceptor binding with the membrane of the brain, the suspension (0.25 mg protein) from brain was incubated with constant shaking for 20 min at 23°C with 0.2 nM ^3H -prazosin in a final volume of 0.5 ml containing 50 mM Tris-HCl buffer (pH 7.4) and 10 mM MgCl_2 , with the indicated concentration of unlabelled drugs. In the case of α_2 -adrenoceptor binding, the concentration of ^3H -p-aminoclonidine in the incubation medium of brain was 0.6 nM. After incubation, the reaction was terminated by rapid filtration under a vacuum through GF/C glass fiber filters using an automatic cell harvester (Labomash LH-101, Labo Science). After 3 times washing of the filters with 1 ml of the incubation medium, the radioactivity remaining in the filters was suspended in 5 ml of Aquazole-2 (New England Nuclear Co., Ltd.), and counted in a liquid scintillation counter (LSC-1000, Aloka Co.). The specific bindings of ^3H -prazosin and ^3H -p-aminoclonidine to receptors in the brain were defined as the difference between the total binding and nonspecific binding, which were determined in the presence of 0.1 mM phenylephrine (α_1 -adrenoceptor agonist) or 0.1 mM clonidine (α_2 -adrenoceptor agonist). The equilibrium dissociation

constant (K_D) and the maximal number of binding sites (B_{max}) were obtained through a Scatchard plot, and the inhibition constant (K_i) values were calculated according to the equation of Cheng and Prusoff (15).

In vivo studies

Systemic blood pressure (SBP) was measured from the right carotid artery with a Nihon Kohden pressure transducer (MPU-0.5). HR changes were monitored with a Nihon Kohden heart rate counter (AT-600G). All recordings were made on charts with a Watanabe linearcorder (model WR 3101). The body temperature of the anesthetized rat was kept at approximately $37 \pm 1^\circ\text{C}$ by a Aquamatic K heating pad (model K-20, Hamilton). For i.v. administration, the femoral vein was cannulated, and 0.2 ml of the drug solutions was injected over a period of approximately 10 sec and then flushed in with 0.9% saline.

Anesthetized rats: Male SHR of Kyoto Wistar origin (300–350 g, 20 weeks of age) from a commercial supplier (Japanese Charles River Labs.) were utilized. Under pentobarbital-Na anesthesia (50 mg/kg, i.p.), the rats were placed in a Narishige stereotactic apparatus with the head fixed to 45° . A stainless-steel needle (0.3 mm in O.D.) was implanted into the lateral cerebroventricle, and the tip was positioned at the following coordinates: 0.8 mm posterior to the bregma, 1.5 mm lateral to the bregma, and 3.6 mm below the surface of the dura mater. The needle was fixed to the skull with dental cement and attached to an extension polyethylene tubing (PE-10), which was filled with the drug and connected to a Terumo microsyringe (model MS-P10). The drugs were given i.c.v. by means of a microsyringe in a volume of 10 μl over a period of 10 sec through the needle cannula. After termination of the experiments, 5 μl of 0.5% thionine was injected i.c.v. The brain was removed and the location of the needle tracks was examined microscopically.

Pithed rats: Male Wistar-Imamichi strain rats (about 350 g) were anesthetized with thiobarbital-Na (50 mg/kg, i.p.). Thirty min later, the animals were pithed in one of two ways. Artificial ventilation was accomplished with a Sinano respirator (SN-480-7) using

100% O_2 in a tidal volume of 1 ml/100 g body weight at a rate of 60 breaths/min. To examine the pressor response to i.v. phenylephrine, rats were completely pithed according to the method of Gillespie et al. (16) by inserting a steel rod through the left orbit and foramen magnum down into the spinal canal. The left and right vagus nerves were cut and the left carotid artery was ligated. The remaining ones were partly pithed according to the procedure of Skarsfeldt and Hyttel (17) by first inserting a metal bar with a diameter of 1.5 mm through the orbit and the foramen magnum and down into the spinal column to the level of the sixth thoracic vertebra. Both the left and the right vagus nerves were cut. To examine the effect of various α_1 -adrenoceptor antagonists on the flexor reflex enhanced by St 587, a central α_1 -adrenoceptor agonist (18, 19), the distal tendon of the anterior tibial muscle of the left hind limb was exposed and the transverse ligament was cut. The tendon of the muscle was attached to a Nihon Kohden isometric force transducer (model SB-IT) with a tension of 7 g. The hind limb was finally fixed to the operating table. According to the procedure described by Skarsfeldt and Hyttel (17), reserpine (10 mg/kg) was injected subcutaneously (s.c.) 24 hr before the experiment. Nialamide (50 mg/kg) was given s.c. 1 hr before the s.c. injection of St 587 (10 mg/kg). A combination of reserpine, nialamide and St 587 produced a marked increase of the electrically induced flexor reflex. Two platinum electrodes were attached to the hind limb. The active electrode was placed between the tibia and the achilles tendon. The indifferent electrode was inserted in the skin between the second and the third digit. Electrical stimulation was performed with rectangular pulses of 20 V, lasting 40 msec twice/min, by means of a Nihon Kohden electronic stimulator (model SEN-7103). The electrical stimulation induced a muscle contraction which was referred to the initial level. Subcutaneous injection of St 587 enhanced the initial level of the reflex. After the increased reflex had reached a maximum and remained steady for at least 10 min, the mean tension of 5 consecutive muscle contractions was calculated

and referred to the basal level. A test substance was then injected i.v. in doses which were increased every 20 min by a factor of 3. The inhibition was measured as the mean of 5 consecutive reflexes between 12.5 and 15 min after the previous injection of the test substance and expressed as percent inhibition of the basal level.

Drugs

The drugs used were: 3-[2-[4-(O-methoxyphenyl)-1-piperazinyl]ethyl]-2,4(1H, 3H)-quinazolin-2-one monohydrochloride (SGB-1534, $C_{21}H_{24}N_4O_3 \cdot HCl \cdot 1/2H_2O$, M.W. 425.9), prazosin hydrochloride (M.W. 419.9) and [2-(2-chloro-5-trifluoromethylphenyl-imino)-imidazolidine] (St 587, M.W. 326.7) (all synthesized by Dr. H. Nagano in our Research Labs.). Other compounds and their sources were: reserpine, nialamide, clonidine hydrochloride, yohimbine hydrochloride and l-phenylephrine hydrochloride (all Sigma) and d,l-noradrenaline hydrochloride (Sankyo). Prazosin, yohimbine and St 587 were dissolved in distilled water to give a concentration of 0.3 mg/ml and diluted with 0.9% saline solution. Reserpine and nialamide were suspended in 2% gum arabic solution and given s.c. in a volume of 1 ml/kg. The other drugs were dissolved in or diluted with 0.9% saline solution to the desired concentrations.

Statistical analysis

Data are expressed as the mean $\pm$ S.E. Intergroup differences were analyzed by Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

Results

Effects of SGB-1534, prazosin and yohimbine on contractile responses of isolated rat aortae to phenylephrine and clonidine: SGB-1534, prazosin and yohimbine in the concentrations used in the present experiment did not affect resting

tension in the aorta.

Phenylephrine or clonidine added to the organ bath resulted in reproducible and concentration-related muscle contractions. Maximal developed tensions induced by phenylephrine and clonidine were 1.61 ± 0.13 g ($n=15$) and 0.134 ± 0.013 g ($n=5$), respectively. Curves of cumulative concentration-response to phenylephrine (3×10^{-9} – 10^{-5} M, each concentration given until stabilization of the response) were obtained before and 10 min after the administration of SGB-1534 (10^{-9} M), prazosin (10^{-8} M) or yohimbine (10^{-6} M). These drugs caused a parallel shift to the right of the concentration-response curve to phenylephrine (Fig. 1). As shown in Table 1, the potency of SGB-1534 in antagonizing the contractile response to phenylephrine was approximately 3.9

Fig. 1. Cumulative molar (M) concentration-response curves for contractile responses of rat aortae to phenylephrine in the absence (●) or the presence (○) of (A) SGB-1534, (B) prazosin or (C) yohimbine. Vertical bars represent the mean $\pm$ S.E. of 5 experiments. The plateau level of contractile response to phenylephrine was taken as 100%.

Table 1. pA_2 values of antagonistic effects of SGB-1534, prazosin and yohimbine to phenylephrine in rat aorta segment preparations

Antagonists	n	pA_2 Values (mean $\pm$ S.E.)
SGB-1534	5	10.57 ± 0.06
Prazosin	5	9.98 ± 0.07
Yohimbine	5	7.43 ± 0.08

times higher than that of prazosin.

Binding property of SGB-1534 to α_1 - and α_2 -adrenoceptors in rat brain: Direct assessment of the binding of SGB-1534 to α_1 - and α_2 -adrenoceptors was determined by competition with ^3H -prazosin and ^3H -p-aminoclonidine, respectively, for specific binding sites in a membrane fraction prepared from the brain. Specific binding of ^3H -prazosin and ^3H -p-aminoclonidine to this membrane fraction was saturable and of high affinity. The ratios of the specific binding to the total one of ^3H -prazosin and ^3H -p-aminoclonidine were $77.2 \pm 2.4\%$ and $86.1 \pm 0.8\%$ ($n=4$), respectively. Scatchard analyses of ^3H -prazosin and ^3H -p-aminoclonidine in the membrane fraction indicated a single class of binding sites. The equilibrium dissociation constant (K_D) and maximal number of specific binding sites ($B_{\max}$) of ^3H -prazosin were 0.16 ± 0.04 (nM) and 28.8 ± 8.6 (fmol/mg protein) ($n=3$), respectively. The K_D and $B_{\max}$ values of ^3H -p-aminoclonidine binding sites were found to be 0.35 ± 0.09 (nM) and 61.5 ± 23.4 (fmol/mg protein) ($n=3$). Table 2 summarizes the negative logarithm of the inhibition constant values (pK_i) of SGB-1534, prazosin and clonidine derived from the displacement experiments. A high pK_i value of SGB-1534 to the α_1 -adrenoceptor was observed, and this value was almost the same as that of prazosin. On the other hand, SGB-1534 and prazosin, compared with clonidine, were much weaker in the displacement effects regarding α_2 -adrenoceptor binding to the brain, as shown in the pK_i values.

Effects of i.v. or i.c.v. SGB-1534, prazosin and clonidine on systemic blood pressure (SBP) and heart rate (HR) in anesthetized SHR: After completion of the surgical procedure, animals were left for at least 30 min

to allow stabilization of SBP and HR.

Single i.v. injection of SGB-1534 (0.3 – $3 \mu\text{g/kg}$) and prazosin (3 – $30 \mu\text{g/kg}$) caused a dose-dependent decrease in SBP, accompanied sometimes by a slight bradycardia (Table 3). The depressor response to SGB-1534 was characterized by a long-duration. At a dose of $1 \mu\text{g/kg}$ of SGB-1534, it reached a maximum (-24.0 ± 6.6 mmHg, $n=5$) within 10 min after the administration and lasted for more than 2 hr. A similar depressor response, in terms of the magnitude (-27.4 ± 2.8 mmHg, $n=5$) and duration (over 2 hr), was observed with a single i.v. injection of prazosin ($30 \mu\text{g/kg}$). Bolus i.v. injection of clonidine (3 – $30 \mu\text{g/kg}$) caused a transient rise, followed by a long-lasting fall of SBP, concomitant with a decrease in HR.

Single i.c.v. injection of SGB-1534 (0.1 – $0.3 \mu\text{g/kg}$) and prazosin (1 – $3 \mu\text{g/kg}$) produced a dose-related hypotension. Clonidine (0.1 – $0.3 \mu\text{g/kg}$) injected i.c.v. also induced a dose-dependent decrease in SBP, but a transient SBP rise was not observed. Original tracings are depicted in Fig. 2. These drugs did not significantly affect HR. The pressor response to noradrenaline ($1 \mu\text{g/kg}$) given i.v. was significantly inhibited by the i.c.v. application of SGB-1534 ($0.3 \mu\text{g/kg}$) and prazosin ($3 \mu\text{g/kg}$) (Table 4). However, in the lesser doses, SGB-1534 ($0.1 \mu\text{g/kg}$) and prazosin ($1 \mu\text{g/kg}$) given i.c.v. did not attenuate the response to i.v. noradrenaline ($1 \mu\text{g/kg}$), although these drugs administered i.v. significantly reduced it. When compared with respect to the doses required to elicit a depressor response of similar magnitude, SGB-1534 was 10–30 times more potent than prazosin by both the i.v. and i.c.v. administrations to the rat. There was no significant changes in SBP and HR after i.v. or i.c.v. administration of

Table 2. Inhibition of ^3H -prazosin and ^3H -p-aminoclonidine binding to α_1 - and α_2 -adrenoceptors in rat brain by SGB-1534, prazosin and clonidine

Drugs	^3H -Prazosin binding (pK_i)	^3H -p-Aminoclonidine binding (pK_i)
SGB-1534	8.81 ± 0.19 ($n=4$)	4.97 ± 0.06 ($n=4$)
Prazosin	9.04 ± 0.22 ($n=4$)	4.71 ± 0.05 ($n=4$)
Clonidine	5.43 ± 0.05 ($n=4$)	8.67 ± 0.08 ($n=4$)

Values are the mean $\pm$ S.E. The concentrations of ^3H -prazosin and ^3H -p-aminoclonidine used were 0.2 and 0.6 nM, respectively.

Table 3. Changes in systemic blood pressure (SBP) and heart rate (HR) after intravenous (i.v.) or intra-cerebroventricular (i.c.v.) injection of SGB-1534, prazosin and clonidine in anesthetized spontaneously hypertensive rats (SHRs)

Drugs	Dose (μ g/kg)	Route	n	SBP (mean, mmHg)		HR (beats/min)	
				Pretreatment value	Maximum Δ changes	Pretreatment value	Maximum Δ changes
SGB-1534	0.3	i.v.	5	184.0 $\pm$ 8.9	-3.0 $\pm$ 1.2	386.0 $\pm$ 19.6	-2.0 $\pm$ 2.0
	1		5	182.6 $\pm$ 9.5	-24.0 $\pm$ 6.6	392.0 $\pm$ 19.8	+0.2 $\pm$ 3.6
	3		5	185.6 $\pm$ 7.1	-38.8 $\pm$ 10.2	402.0 $\pm$ 13.6	-18.6 $\pm$ 12.1
	0.1	i.c.v.	5	179.0 $\pm$ 5.3	-5.0 $\pm$ 2.2	398.0 $\pm$ 13.6	-1.0 $\pm$ 4.0
	0.3		5	178.0 $\pm$ 5.8	-24.2 $\pm$ 5.5	400.0 $\pm$ 14.5	-11.6 $\pm$ 7.5
Prazosin	3	i.v.	5	190.0 $\pm$ 1.6	-9.0 $\pm$ 2.9	368.0 $\pm$ 30.2	+2.0 $\pm$ 6.2
	10		5	191.0 $\pm$ 1.9	-18.6 $\pm$ 2.7	366.0 $\pm$ 27.5	-2.0 $\pm$ 3.7
	30		5	190.0 $\pm$ 2.7	-27.4 $\pm$ 2.8	364.0 $\pm$ 26.9	-24.4 $\pm$ 7.6
	1	i.c.v.	5	177.0 $\pm$ 4.9	-4.0 $\pm$ 2.4	354.0 $\pm$ 18.1	-6.0 $\pm$ 2.4
	3		5	180.0 $\pm$ 5.2	-18.0 $\pm$ 4.1	348.0 $\pm$ 18.3	-9.0 $\pm$ 5.6
Clonidine	3	i.v.	5	175.0 $\pm$ 8.4	+21.2 $\pm$ 3.7, -11.6 $\pm$ 3.5	324.0 $\pm$ 23.8	-41.6 $\pm$ 8.2
	10		5	173.6 $\pm$ 5.1	+13.8 $\pm$ 2.6, -26.4 $\pm$ 3.9	445.0 $\pm$ 14.1	-54.0 $\pm$ 11.1
	30		5	178.6 $\pm$ 2.2	+28.6 $\pm$ 5.7, -69.4 $\pm$ 6.4	437.0 $\pm$ 12.8	-124.0 $\pm$ 8.3
	0.1	i.c.v.	5	178.6 $\pm$ 8.4	-9.6 $\pm$ 6.0	352.0 $\pm$ 19.3	-1.0 $\pm$ 5.1
	0.3		5	183.0 $\pm$ 7.7	-42.8 $\pm$ 12.0	394.0 $\pm$ 17.5	-31.0 $\pm$ 17.8

Each value represents the mean $\pm$ S.E.

Fig. 2. Effects of intravenous (i.v.) and intracerebroventricular (i.c.v.) injection of SGB-1534, prazosin and clonidine on systemic blood pressure (SBP) in anesthetized SHR.

Table 4. Effects of intravenous (i.v.) and intracerebroventricular (i.c.v.) injection of SGB-1534 and prazosin on mean systemic blood pressure responses to 1 µg/kg noradrenaline (NA) administered i.v. in anesthetized SHR

Drugs	Dose (µg/kg)	Peak pressor response to 1 µg/kg NA			
		i.v.		i.c.v.	
		pretreatment	after treatment	pretreatment	after treatment
		mmHg		mmHg	
0.9% Saline		42.6±4.2	42.6±3.5	38.0±5.5	36.6±4.8
SGB-1534	0.1	46.6±1.4	31.6±1.4**	40.2±2.5	47.0±3.4
	0.3			46.4±4.2	28.0±3.0**
Prazosin	1	42.6±3.5	19.2±1.7**	41.4±2.2	48.0±6.6
	3			35.2±4.6	22.0±2.3*

Vertical bars represent the mean±S.E. of 5 experiments. *P<0.05, **P<0.01, compared to pretreatment values. The depressor response to SGB-1534 and prazosin reached a peak approximately 5 min after the i.v. or i.c.v. administration. Then 1 µg/kg NA was given i.v.

0.9% saline.

Effects of SGB-1534, prazosin and yohimbine given i.v. on the St 587-enhanced flexor reflex in pithed rats: After completion of the surgical procedure, the preparations were left for at least 60 min in order for them to become free from anesthesia. Electrical stimulation resulted in a muscle tension of 16.3±1.1 g (n=15) (initial level). St 587 given s.c. in a dose of 10 mg/kg elicited an increase of the initial level of the flexor reflex by 41.1±4.0 g (n=15). Single i.v. injection of SGB-1534 (0.1–10 mg/kg), prazosin (0.03–3 mg/kg) and yohimbine (3–30 mg/kg) were made in a cumulative manner at 20 min intervals. These drugs inhibited the St 587-enhanced flexor reflex

in a dose-dependent fashion (Fig. 3A and B). When compared on the dose producing a 50% inhibition (ID₅₀) of the sustained phase of the St 587-enhanced flexor reflex (Table 5), the antagonistic activity of SGB-1534 was 15 times less potent than prazosin on a weight basis. In preliminary experiments, it was confirmed that the flexor reflex response remained stable for over 3 hr after St 587 administration.

Effects of SGB-1534, prazosin and yohimbine administered i.v. on the pressor response to phenylephrine given i.v. in pithed rats: When phenylephrine (1 µg/kg) was administered i.v. repeatedly at 15 min intervals before and over 2 hr after 0.9% saline i.v. injection, the magnitude of the

Fig. 3. Effects of intravenous (i.v.) injection of SGB-1534, prazosin and yohimbine on St 587 (10 mg/kg, s.c.)-enhanced flexor reflex in pithed rats. A) Original tracings. B) Summarized data. Vertical bars represent the mean $\pm$ S.E. of 5 experiments. SGB-1534, prazosin and yohimbine are given i.v. in a cumulative manner.

Table 5. ID₅₀ values of antagonistic effects of SGB-1534, prazosin and yohimbine to St 587 and phenylephrine in pithed rats

Antagonists	n	Effect on St 587-enhanced flexor reflex ID ₅₀ (mg/kg)	Effect on pressor response to phenylephrine ID ₅₀ (μ g/kg)
SGB-1534	5	10.6 $\pm$ 4.9	0.66 $\pm$ 0.08
Prazosin	5	0.7 $\pm$ 0.2	1.06 $\pm$ 0.15
Yohimbine	5	36.6 $\pm$ 16.5	182.4 $\pm$ 54.7

Each value represents the mean $\pm$ S.E.

pressor response to phenylephrine virtually remained unchanged throughout the experimental period (not shown). The peak increase in diastolic SBP was 41.4 $\pm$ 3.4 mmHg (n=15). The pressor effect of phenylephrine was significantly attenuated after i.v. treatment with SGB-1534 (0.1–10 μ g/kg), prazosin (0.1–10 μ g/kg) and yohimbine (0.01–1 mg/kg) in a dose-dependent manner (Fig. 4). These α -adrenoceptor antagonists did not affect the basal level of SBP and HR. As shown in Table 5, the α_1 -adrenoceptor antagonistic activity of SGB-1534 was approximately 1.6

times greater than that of prazosin on a weight basis.

Discussion

The present study demonstrated that SGB-1534 in i.c.v. as well as i.v. administration to anesthetized SHR produced a dose-dependent and long-lasting hypotensive response associated with very few changes in HR. Similar results were observed with prazosin. It should be noted that i.c.v. injection of the two drugs at a dosage 3 times less than that in the i.v. injection elicited a

Fig. 4. Dose-response curves for suppression of the pressor responses to phenylephrine (1 μ g/kg, i.v.) by i.v. administration of SGB-1534, prazosin and yohimbine in pithed rats. Vertical bars represent the mean $\pm$ S.E. of 5 experiments. SGB-1534, prazosin and yohimbine were injected i.v. at 15 min intervals in a cumulative way. The pressor response to phenylephrine is measured as an increase in diastolic blood pressure and expressed as a percent inhibition of the pressor response to phenylephrine before the treatment with α -adrenoceptor antagonists. Baseline level of diastolic SBP, 50.2 ± 2.8 mmHg ($n=15$).

decrease in SBP in a similar magnitude. Thus, one might argue that the depressor response to SGB-1534, like prazosin, is dependent on the central sympathetic nervous system. Actually, there have been some reports that the central α_1 -adrenoceptor component may participate in the hypotensive action of prazosin (20–22). Dashwood (23), however, stated that prazosin does not readily pass the blood-brain barrier (BBB), and no specific prazosin binding could be seen in the thoracic spinal cord of the rat. Recently, Luft et al. (24) also demonstrated that prazosin given i.c.v. and i.v. to conscious, chronically instrumented stroke-prone SHR, unlike clonidine, does not affect efferent splanchnic sympathetic nerve activity, and they claimed that prazosin lowers blood pressure through peripheral blockade of α_1 -adrenoceptors rather than by means of central sympathetic suppression. Thus, the observations with prazosin still remain controversial.

It should also be noted that SGB-1534 injected i.c.v., like prazosin, significantly attenuated the pressor response to nor-adrenaline injected i.v. Furthermore, nearly equal doses of SGB-1534 and prazosin given

i.c.v. and i.v. elicit the hypotensive effect in a similar magnitude, even though the i.v. doses of the drugs were somewhat higher than the i.c.v. ones. These findings indicate that SGB-1534 and prazosin applied i.c.v. may have leaked out of the ventricular space to the peripheral circulation, and antagonism may have occurred at peripheral sites. In fact, it was postulated that prazosin given i.c.v. moves readily from the cerebrospinal fluid (CSF) to the blood stream by diffusing across the blood-CSF barrier or passes across the arachnoid villi from the CSF (25). Thus, it was not clear whether the hypotensive effect of SGB-1534 as well as prazosin given by a peripheral route (i.v.) has any central component.

In view of this, it was of interest to examine whether SGB-1534 as well as prazosin antagonizes the St 587-enhanced flexor reflex which has been proposed as a model for assessment of antagonism at α_1 -adrenoceptors in the central nervous system (17). In short, a combination of reserpine, nialamide and St 587 induces a marked increase of the electrically induced flexor reflex in pithed rats. This increased reflex is significantly inhibited by the α_1 -adrenoceptor antagonist prazosin, but not by the α_2 -adrenoceptor antagonist idazoxan. Furthermore, an inhibition of the St 587-enhanced reflex shows a close correlation to α_1 -receptor affinities in vitro, while no correlation was found to either D-2 receptor affinities or to 5-HT₂ receptor affinities in vitro. In the present study, SGB-1534 given i.v. in pithed rats, like prazosin, dose-dependently inhibited the St 587-enhanced flexor reflex. However, the activities of SGB-1534 and prazosin in inhibiting the St 587-enhanced flexor reflex were 16,000 and 660 times, respectively, less than those in attenuating the pressor response to i.v. phenylephrine in the pithed rats. Thus, it appears that the BBB is much more impermeable to SGB-1534 than to prazosin.

The present experiments confirm that in isolated rat aortae, SGB-1534, as compared with prazosin, possesses a stronger α_1 -adrenoceptor antagonistic activity, in agreement with the previous results (6–9). Additionally, in a ³H-ligand binding assay using rat brain, SGB-1534 had a highly specific

affinity, comparable to that of prazosin, to α_1 -adrenoceptor binding sites but not to α_2 -adrenoceptor binding sites. This finding in rat brain agrees well with that reported recently by Nagatomo et al. (26) in a ^3H -ligand binding assay using canine brain and aorta. Thus, SGB-1534 is surely a potent and selective α_1 -adrenoceptor antagonist. Therefore, if SGB-1534 readily penetrates the BBB to gain access to the cardiovascular regulatory centers in the medulla, SGB-1534 should have markedly inhibited the St 587-enhanced flexor reflex in pithed rats.

Certainly, the present study does not apparently indicate to what extent i.v. SGB-1534 and prazosin act centrally to lower SBP. However, in our recent experiments using anesthetized dogs, the SBP lowering activities of SGB-1534 and prazosin were significantly correlated to the α_1 -adrenoceptor antagonism produced by these drugs in the renal vasculature (9). This close relationship indicates that the hypotensive effects of SGB-1534 greatly depend on the peripheral α_1 -adrenoceptor blockade. Furthermore, Matsumoto et al. (27) evidenced in the experiment using anesthetized rats that a hypotensive dose of SGB-1534 (0.5 mg/kg, i.v.) does not affect the sympathetic adrenal nerve activity, suggesting that SGB-1534 lacks a central effect. Actually, it has been recently noted that from 20 min to 24 hr after oral dosing of 1 mg/kg of ^{14}C -SGB-1534 (an effective hypotensive dose in SHR (5)) to rats, the radioactivity is hardly detectable in the brain (prepared for publication).

In summary, SGB-1534 is a highly selective and potent α_1 -adrenoceptor antagonist. It appears that SGB-1534, by the i.v. route, does not easily penetrate the BBB, and therefore indicates that the hypotensive effect of SGB-1534 mainly depends on the peripheral α_1 -adrenoceptor blockade rather than the central nervous system.

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