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Effect of 3-[2-[4-(*o*-Methoxyphenyl)-1-piperazinyl]ethyl]-2,4(1*H*,3*H*)-quinazolinedione Monohydrochloride (SGB-1534), an Antihypertensive Agent, on ^{3}H-Prazosin and ^{3}H-*p*-Aminoclonidine Binding to α_1- and α_2-Adrenoceptors in Dog Brain and Aorta

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In order to assess the antihypertensive activities of SGB-1534, 3-[2-[4-(*o*-methoxyphenyl)-1-piperazinyl]ethyl]-2,4-(1*H*,3*H*)-quinazolinedione monohydrochloride, the effect of this drug on the ^{3}H-prazosin, ^{3}H-*p*-aminoclonidine and ^{3}H-dihydroalprenolol (^{3}H-DHA) bindings to α_1-, α_2- and β-adrenoceptors in dog brain and aorta was determined by means of radioligand binding assay and compared with that of prazosin. Potent inhibition of ^{3}H-prazosin binding to α_1-adrenoceptor sites in the brain and the aorta by SGB-1534 was observed (the potency in the brain was almost the same as that of prazosin), but the inhibition of ^{3}H-*p*-aminoclonidine and ^{3}H-DHA bindings to α_2- and β-adrenergic binding sites by both SGB-1534 and prazosin was very weak. In addition, a high pA_2 value of α_1-blocking action by SGB-1534 was found in a pharmacological experiment. Thus, these results suggest that the inhibitory effect of SGB-1534 at α_1-adrenoceptor binding sites, but not at α_2- or β-adrenoceptor sites, may contribute to the hypotensive effect.

Keywords——α_1-adrenoceptor antagonist; α_2-adrenoceptor; SGB-1534; dog; aorta; ^{3}H-prazosin; ^{3}H-*p*-aminoclonidine

Introduction

Sakai *et al.*[1] reported that SGB-1534, 3-[2-[4-(*o*-methoxylphenyl)-1-piperazinyl]ethyl]-2,4(1*H*,3*H*)-quinazolinedione monohydrochloride, showed potent and long-lasting hypotensive effects on hypertensive rats or miniature pigs. In fact the intravenous administration of this drug apparently induced a sustained and systemic decrease of blood pressure in anesthetized miniature pigs. These observations suggest that this hypotensive effect is probably due to peripheral vasodilation, not myocardial depression.

It is well known that α_1-adrenoceptors in peripheral vessels contribute to the regulation of peripheral vascular resistance and are of major clinical importance as a site of action of antihypertensive agents. Our previous paper[2] and others[3] have demonstrated the existence of α-adrenergic receptors in vascular smooth muscles by using the radioligand binding assay. In addition, the method reported by us[2] could be useful in the assessment of newly synthesized chemicals as α-adrenergic antagonists. On the other hand, prejunctional α_2-receptors are believed to modulate the release of norepinephrine from sympathetic nerve terminals by a negative feed-back mechanism.[4] There are also data suggesting the existence of a postjunctional α_2-receptor, which can be activated by α_2-agonists to produce vasoconstriction in pithed animals.[5] It is of interest to test the action of SGB-1534 in the dog blood vessel in addition to examining the existence of α_2-adrenoceptors in blood vessels by using the radioligand binding assay method. ^{3}H-*p*-Aminoclonidine has been an excellent probe for the study of α_2-adrenoceptors, and it binds with a greater affinity than ^{3}H-clonidine to those

sites.[6] Thus, in the present study, the effects of SGB-1534 on the ^{3}H-prazosin, ^{3}H-*p*-aminoclonidine or ^{3}H-dihydroalprenolol (^{3}H-DHA) binding to α_1-, α_2- or β-adrenoceptors in dog brain and aorta were examined.

Methods

Radioligands——^{3}H-Prazosin (30 Ci/mmol), ^{3}H-*p*-Aminoclonidine (50 Ci/mmol) and ^{3}H-DHA (104.8 Ci/mmol) in ethanol were purchased from the New England Nuclear Corporation, Ltd. and stored at −20 °C. These isotopes were diluted to appropriate concentrations with double distilled water before use.

Isolation of Membrane Fraction——The isolation of membrane fraction from dog brain and aorta was carried out by using the method previously reported by us.[2] In brief, mongrel dogs of either sex were anesthetized with sodium thiopental (30 mg/kg) administered intravenously, then the brain and the descending aorta were quickly removed, frozen in liquid nitrogen and stored in a freezer (−80 °C) until use. As described in the previous report, frozen aorta was crushed into a fine powder in liquid nitrogen using a mortar and pestle. A weighed portion of the powdered tissue was defrosted at room temperature and this fine powdered tissue was suspended in 10 volumes of 0.25 M sucrose, 1 mM $MgCl_2$, 5 mM Tris–HCl buffer, pH 7.4. This suspension was homogenized in a Polytron homogenizer three times for 10 s at a setting of 8. After being filtered through one layer of gauze, the homogenate was subjected to stepwise differential centrifugation at 4 °C, first at $1000 \times g$ for 10 min to remove the cell debris, nuclei and collagen, and then at $40000 \times g$ for 30 min. The pellets obtained were resuspended in an incubation buffer (100 mM Tris–HCl buffer, pH 7.5, and 20 mM $MgCl_2$) and used for the radioligand binding assay.

The dog cerebral cortex was also defrosted at room temperature, then suspended in 10 volumes of ice-cold 0.25 M sucrose, 5 mM Tris–HCl buffer, pH 7.5, and minced with small scissors. The homogenization was carried out using a glass homogenizer in 10 vol. (w/v) of the above buffer. The homogenate was filtered through 4 layers of gauze and the suspension was centrifuged at $40000 \times g$ for 30 min. The pellets were resuspended in an incubation buffer containing 100 mM Tris–HCl buffer, pH 7.5, and 20 mM $MgCl_2$. Protein was determined by using the method of Lowry *et al.*[7]

Binding Studies——The membrane suspension was incubated at 23 °C with the indicated concentration of ^{3}H-prazosin or ^{3}H-*p*-aminoclonidine (see Table and Figure) in an incubation medium containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, with or without unlabelled drugs. The reaction was started by adding 0.25 mg of membrane suspension to make a final volume of 1 ml (aorta) or 0.5 ml (brain). After 60 min of incubation, the reaction was terminated by rapid filtration under vacuum through GF/C glass fiber filters using an automatic cell harvester (Labomash LM-101, Labo Science, Tokyo, Japan). The filters were washed with 1 ml of the incubation medium, then the radioactivity remaining on the filters was counted using a scintillation counter. The specific binding of ^{3}H-prazosin or ^{3}H-*p*-aminoclonidine to α_1- and α_2-adrenoceptors in the brain and the aorta was defined as the difference between the total binding and the nonspecific binding, which were determined in the presence of 0.1 mM phentolamine or clonidine. The ^{3}H-DHA binding studies for β-adrenoceptors in brain were carried out by the method described in our previous paper.[8] Equilibrium dissociation constants (K_d) and maximal binding number (B_{max}) were analyzed by using Scatchard plots and values of K_i were calculated according to the equation described in the previous paper.[8]

Assessment of the α-Blocking Action: The pA_2 value of α_1-blocking action was obtained by using the method described previously.[2] The aorta of a male Wistar rat weighing 250—350 g was cut into rings approximately 2 mm in width and the preparation was mounted in a 12 ml organ bath. The bathing solution was Krebs–Henseleit's solution of the following composition: NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25.0 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM and glucose, 12 mM. The temperature of the solution was maintained at 37 ± 1 °C and was aerated with a mixture of 95% O_2 + 5% CO_2. The contractile tension of the aorta was recorded on a potentiometric recorder (Hitachi APD-74) using a strain gauge transducer and a carrier amplifier (Nihon Kohden AP-620G). The aorta was always stretched to an appropriate length with a load of 0.5—1 g to get optimum responses. Dose response curves were determined using phenylephrine before and after addition of each of the drugs.

Chemicals——SGB-1534 used in the present study was donated by Chugai Pharmaceutical Co., Ltd.

Results

The protein yields of the membrane fraction prepared from the brain and aorta were 69.99 ± 11.82 (mean ± S.E., $n=5$) and 4.41 ± 0.91 (mean ± S.E., $n=5$) mg/g tissues, respectively. Figures 1 and 2 illustrate the results of the saturation experiments and of Scatchard analysis using ^{3}H-prazosin and ^{3}H-*p*-aminoclonidine. Scatchard analysis of α_1- and α_2-adrenoceptors in both of brain and aorta indicated a single class of binding sites. The value of K_d of α_1-adrenoceptors in the brain was higher than that of the aorta. The K_d of α_2-

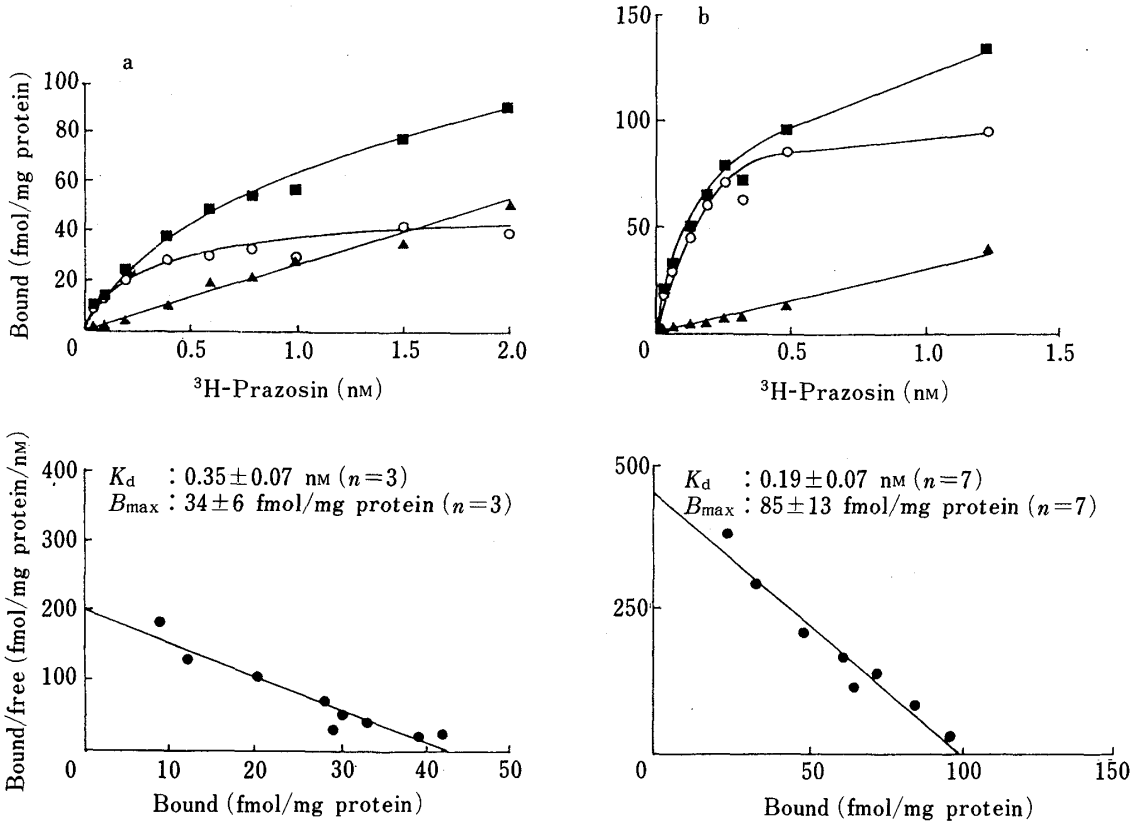

Fig. 1. The Upper Half: Effects of Concentration of ^{3}H-Prazosin on the Amount of ^{3}H-Prazosin Bound to the Dog Brain (a) and Aorta (b) in the Presence (▲) and Absence (■) of 0.1 mM Phentolamine

The Lower Half: Scatchard Plots of the Same Data

The difference of the mean value of the total binding (■) and the non-specific binding (▲) was taken as the specific binding (○). The concentration of ^{3}H-prazosin ranged from 0.01 to 2.0 nM (brain) and 1.25 nM (aorta).

Each value is the mean±S.E.

TABLE I. Inhibition of ^{3}H-Prazosin and ^{3}H-*p*-Aminoclonidine Bindings to α_1- and α_2-Adrenoceptors in Dog Brain and Aorta by SGB-1534

	^{3}H-Prazosin binding		^{3}H-*p*-Aminoclonidine binding	
	Brain K_i (nM)	Aorta K_i (nM)	Brain K_i (nM)	Aorta K_i (nM)
SGB-1534	1.45±0.58 (4)	1.06±0.50 (4)	6040±1300 (4)	169000±117000 (3)
Prazosin	1.29±0.55 (4)	0.40±0.32 (6)	21300±6390 (4)	115000±40200 (3)
Clonidine	89600±88300 (2)	3600±2310 (3)	1.40±0.27 (4)	15.5±4.66 (3)

Each value is the mean±S.E. Numbers in parenthesis represent the numbers of experiments. The concentrations of ^{3}H-prazosin and ^{3}H-*p*-aminoclonidine used were 0.1 (brain) and 0.6 nM (aorta), and 0.6 (brain) and 1 nM (aorta), respectively.

adrenoceptors in the brain for ^{3}H-*p*-aminoclonidine was significantly much lower than that in the aorta ($p<0.05$). The values of B_{max} of α_2-adrenoceptors in the brain and aorta were almost identical.

Table I summarizes the K_i values of SGB-1534 derived from the displacement experiments. A low K_i value of SGB-1534 for α_1-adrenoceptors in the brain was observed and this value was almost the same as that of prazosin. However, the K_i value of SGB-1534 in aorta

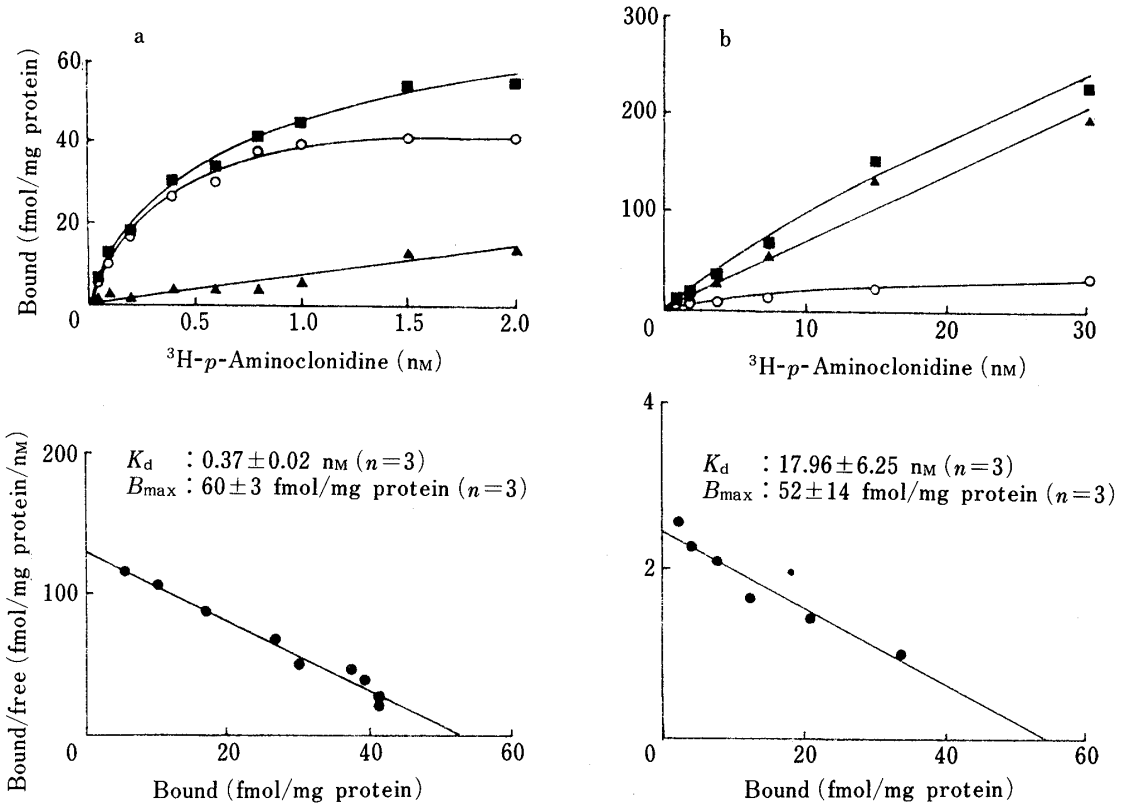

Fig. 2. The Upper Half: Effects of Concentration of ^{3}H-p-Aminoclonidine on the Amount of ^{3}H-p-Aminoclonidine Bound to the Dog Brain (a) and Aorta (b) in the Presence (▲) and Absence (■) of 0.1 mM Clonidine
The Lower Half: Scatchard Plots of the Same Data

The difference of the mean value of the total binding (■) and the non-specific binding (▲) was taken as the specific binding (○). Concentrations of ^{3}H-p-aminoclonidine ranged from 0.05 or 1 to 2.0 (brain) or 30 nM (aorta), respectively.
Each value is the mean±S.E.

TABLE II. Inhibition of ^{3}H-Dihydroalprenolol Binding to β-Adrenoceptors in Brain by SGB-1534

Drugs	K_i
dl-Propranolol (4)	49.4±17.4
SGB-1534 (4)	20900±11800
Prazosin (4)	148300±42800

Each value is the mean±S.E. Numbers in parenthesis represent the numbers of experiments. The concentration of ^{3}H-DHA used was 1.5 nM.

TABLE III. pA_2 Values of SGB-1534 and Prazosin

	pA_2
Prazosin	10.88±0.22 (5)
SGB-1534	9.74±0.30 (5)

Each value is the mean±S.E. The numbers in parentheses represent the number of experiments.

was slightly higher than that of prazosin. Both SGB-1534 and prazosin showed obviously weak displacing effects in α_2-adrenoceptor binding to the brain and the aorta membrane. As shown in Table II, a weak inhibition of ^{3}H-DHA binding to β-adrenoceptors in the brain by SGB-1534 was observed. In addition, high pA_2 values of SGB-1534 and prazosin were obtained by pharmacological methods using rat aorta (Table III).

Discussion

We reported in our previous paper that the radioligand binding assay method using ^{3}H-prazosin as a radioligand could be useful for the assessment of α_1-blocking action of newly synthesized chemicals because there was a good relation between IC_{50} values of α-adrenergic antagonists in the aorta and pA_2 values obtained from the contractile response of the rat aorta to phenylephrine.[2] Thus, the paper suggested that this radioligand binding assay is a good method for the assessment of α-blocking activity. As shown in Table I, SGB-1534 apparently inhibited ^{3}H-prazosin binding to α_1-adrenoceptors in dog aorta and brain. Antihypertensive activities of SGB-1534 were observed in anesthetized or conscious hypertensive rat models or pentobarbital-anesthetized miniature pigs.[1] These *in vivo* and *in vitro* experiments strongly suggest that SGB-1534 is a selective and competitive antagonist of the α_1-adrenoceptors in peripheral vessels. In addition, the potency of SGB-1534 reported here was almost the same as that of prazosin. Thus, the results of the present study suggest that SGB-1534 could be a strong α_1-blocker.

Recent evidence indicates that alterations in central nervous system (CNS) cathecholaminergic mechanisms may be related to the regulation of blood pressure.[9] Also, it is clear that noradrenergic receptors in CNS may be important at the ventrolateral medulla and the intermediolateral column of the spinal cord. It is well known that α_1-adrenergic receptors exist in CNS[10] and that the changes in α_1-adrenoceptor number in the hypothalamus may be important in both the generation and maintenance of high blood pressure.[11] Thus, we suggest that a high IC_{50} value of SGB-1534 in α_1-adrenoceptors in the brain may be an important factor in the antihypertensive effects.

It is well known that α-adrenoceptors can be subdivided into postsynaptic α_1-receptors that mediate contraction of smooth muscle and presynaptic α_2-receptors that mediate a reduction of the action potential-induced output of the transmitter from adrenergic nerve terminals. However, adrenoceptors of α_2-subtype on the postsynaptic membranes apparently activate to produce vasoconstriction in pithed animals.[5,12] As ^{3}H-*p*-aminoclonidine has been an important radioligand for the study of α_2-adrenoceptors and this radioligand shows greater affinity than ^{3}H-clonidine to α_2-adrenoceptors,[6] the present study used this radioligand and showed that ^{3}H-*p*-aminoclonidine definitely binds to α_2-adrenoceptors in both dog brain and aorta. However, a higher affinity of this radioligand was observed in the brain than in the aorta (Fig. 2). The values of K_d and B_{max} of the aorta presented here are in agreement with those obtained from rat mesenteric artery[13] even though ^{3}H-yohimbine was used in those experiments as a radioligand for α_2-binding sites. Weiss *et al.*[14] also suggested that the specific binding of the α_2-agonist (^{3}H-clonidine) to the rat tail artery smooth muscle membrane confirmed the presence of a post-junctional α_2-adrenoceptor. The present study showed that the inhibition of ^{3}H-*p*-aminoclonidine binding to α_2-adrenoceptors in dog aorta by both SGB-1534 and prazosin was very weak. Therefore, these results imply that these chemicals do not interact with α_2-adrenoceptors in peripheral vascular smooth muscles, indicating that SGB-1534 is not concerned with the vasoconstriction or vasodilation through α_2-adrenoceptors.

Although the details of most of the mechanisms and of the synaptic location of the α_2-adrenoceptors are poorly understood because of the complexity of the CNS, clonidine lowers blood pressure *via* a primary central mechanism of action, although this drug exerts

pronounced α_2-adrenoceptor stimulatory properties. Accordingly, the central hypotensive effect of clonidine has been attributed to the excitation of α-adrenoceptors.[12] The inhibition by SGB-1534 of ^{3}H-*p*-aminoclonidine binding to α_2-adrenoceptor sites in the central nervous system was very weak, indicating that the hypotensive effect due to α_2-adrenoceptor activation in the CNS by SGB-1534 may not be important.

In conclusion, the hypotensive effect of SGB-1534 may be due to the α_1-adrenoceptor blocking action and not to α_2- or β-adrenoceptors. This view is supported by the high pA_2 value of SGB-1534 obtained in the pharmacological experiment.

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