Characterization of a Novel $\alpha_1$-Adrenoceptor Antagonist, SGB-1534, in Contractile Response of Isolated Canine Arterial and Venous Smooth Muscle to Exogenous Noradrenaline: Comparison with Prazosin, Phentolamine and Yohimbine

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Abstract—The pharmacological properties of SGB-1534, 3-[2-[4-[(o-methoxyphenyl)-1-piperazinyl]ethyl]-2,4(1H,3H)-quinazolinedione monohydrochloride, a selective $\alpha_1$-adrenoceptor antagonist, compared with prazosin, phentolamine and yohimbine, were examined in contractile responses of isolated canine mesenteric arteries and veins and femoral arteries and veins to exogenous noradrenaline. The arteries and veins concentration-dependently contracted when exposed to noradrenaline. The sensitivity to noradrenaline, when compared in terms of pD₂ values, was significantly higher in the veins than in the arteries. Phentolamine and yohimbine were competitive antagonists against noradrenaline in the arteries and the veins. SGB-1534 and prazosin caused a parallel shift to the right of the concentration-response curves for noradrenaline only in the arteries: the two antagonists were less effective in the veins than in the arteries when low concentrations of noradrenaline were applied. The pharmacological characteristics of SGB-1534 resemble those of prazosin. The pA₂ values for SGB-1534 against noradrenaline in the arteries were much higher than those for prazosin, phentolamine and yohimbine. The result indicates that SGB-1534 may predominantly act upon arterial resistance vessels rather than the venous side, resulting in potent hypotension.

It is well-documented that the principal feature of essential hypertension is an elevation of total peripheral vascular resistance (1–3), under the active control of sympathetic nerve fibers (4–6). Numerous observations have indicated that vasculatures contain two subpopulations of $\alpha$-adrenoceptors, i.e., $\alpha_1$- and $\alpha_2$-adrenoceptors mediating vasoconstrictor responses to $\alpha$-adrenoceptor agonists (7, 8). SGB-1534 is a novel antihypertensive drug currently undergoing clinical evaluation (9). Recently, it has been revealed that SGB-1534 possesses a selective $\alpha_1$-adrenoceptor antagonistic activity in in vitro and in vivo preparations from guinea pigs and rats (10), rabbits and cats (11), and dogs (12). Furthermore, Nagatomo et al. (13) and Aono and Sakai (14) using dogs and rats, respectively, found in recent $^3$H-radioligand experiments that SGB-1534 binds with a highly specific affinity, comparable to that of prazosin, to $\alpha_1$-adrenoceptor sites in the brain and the aorta, but not to $\alpha_2$-adrenoceptor sites. Thus, it appears that SGB-1534 is a potent, selective $\alpha_1$-adrenoceptor antagonist.

This study was therefore undertaken to evaluate the characteristic effects of SGB-1534, compared with the other $\alpha$-adrenoceptor antagonists, prazosin, phentolamine and yohimbine, on the contractile response of the isolated arteries and veins of dogs to exogenously applied noradrenaline.
Materials and Methods

The experiments were carried out on isolated femoral arteries, femoral veins, superior mesenteric arteries, and common mesenteric veins taken from adult beagle dogs (8–15 kg) of both sexes anesthetized with sodium pentobarbital (35 mg/kg, i.v.). Rings of arteries and veins (5–7-mm-wide) were mounted under a resting tension of 10 g for the arteries or 1 g for the veins in a 10 ml organ bath containing a modified Krebs-Henseleit (K-H) solution of the following composition: 119.0 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24.8 mM NaHCO₃, 10.0 mM glucose and 0.057 mM ascorbic acid. In order to block β-adrenoceptors and uptake of catecholamines to nerve endings, 10⁻⁶ M propranolol and 10⁻⁵ M cocaine were added to the K-H solution. The solution was aerated with a gas mixture containing 95% O₂ and 5% CO₂. The oxygen tension of the solution was approximately 600 mmHg, and the pH was 7.4 at 37°C (measured with a blood gas analyzer, ABL2, Acid-Base Laboratory, Radiometer, Copenhagen). One side of the ring preparation was fixed to the bottom of the bath, and the other end was connected by a hook at the lever of a Nihon Kohden force-displacement transducer (TB-611T). Six preparations were run concurrently. Before the commencement of the experiments, all preparations were allowed to equilibrate for at least 1 hr at 37°C, with washes every 20 min. The drugs were added to the bath in a volume of less than 0.1 ml. A series of sequential concentrations was increased by a factor of about 3, each subsequent concentration being introduced when the effect of the preceding one had reached a steady value. Thus, the cumulative concentration-percentage maximal response curve for muscle contraction of noradrenaline was generated. An antagonist was added to the organ bath; and 10 min later, the cumulative concentration-response curve to noradrenaline was constructed for each tissue. To correct for changes in sensitivity with time, control concentration-response curves for noradrenaline were obtained in parallel on preparations from the same arteries and veins. Contractions induced by noradrenaline were expressed as a percentage of the maximum response. Isometric tension was recorded on a Yokogawa self-balancing potentiometric pen recorder (Type 3066).

Drugs used: 3-[2-[4-(o-Methoxyphenyl)-1-piperazinyl]ethyl]-2,4(1H,3H)-quinazolininedione monohydrochloride (SGB-1534, M.W. 416.5, C₂₁H₂₄N₄O₃.HCl) (15) and prazosin hydrochloride (M.W. 419.9) (16) were synthesized in our Organic Chemistry Laboratory. Other compounds and their sources were phentolamine mesylate (Ciba-Geigy), β-noradrenaline bitartrate (Sigma), yohimbine hydrochloride (Sigma), cocaine hydrochloride (Takeda) and /-propranolol hydrochloride (Sumitomo). The drugs were dissolved in distilled water and diluted with 0.9% saline solution. All drug concentrations are expressed as final molar (M) concentrations in the bath solution.

Statistical analysis: Data are presented as the means±S.E. The EC50 value of noradrenaline was determined for each concentration-response curve, and the logarithm \{EC50 value in the presence of the antagonist)/(EC50 value in the absence of the antagonist) - 1 : concentration ratio - 1 \} was plotted against the negative logarithm of the molar concentration of the antagonist (17). Simple competition between an agonist and an antagonist for each receptor predicts a slope of 1.0. The negative logarithm of molar concentrations of antagonists causing a 2-fold shift to the right of the concentration-response curve for noradrenaline (pA₂ values) was determined according to the Schild analysis. The pD₂ values for noradrenaline were also determined from each concentration-response curve as the negative logarithm of the molar concentration causing 50% of the maximum responses to noradrenaline. The data from the noradrenaline concentration-response curve in the presence and absence of the α-adrenoceptor antagonists were subjected to the test for parallelism by a computer program (2×3 assay, Muscot series, Y.D.K. Co., Ltd.). Student's t-test for unpaired observations was used. P values less than 0.05 were considered to be significantly different.
Results

SGB-1534, prazosin, phentolamine and yohimbine, in concentrations used in the present experiment, did not affect resting tension in the femoral artery, the femoral vein, the mesenteric artery and the mesenteric vein. Noradrenaline added to the bath produced reproducible, concentration-dependent contractions in both arteries and veins. Curves of cumulative concentration-response to noradrenaline were determined in the absence or presence of α-adrenoceptor antagonists. The curves were constructed 10 min after the application of SGB-1534 (3×10^{-10}–3×10^{-9} M), prazosin (10^{-8}–10^{-7} M), phentolamine (3×10^{-8}–3×10^{-7} M) or yohimbine (3×10^{-7}–3×10^{-6} M in the arteries, and 10^{-7}–10^{-6} M in the veins) (Figs. 1, 2, 4 and 5). As shown in Table 1, the pD2 value for noradrenaline determined in the absence of any α-adrenoceptor antagonist differed significantly (P<0.001) between the arteries and veins: it was the lowest in the femoral arteries, followed by the mesenteric arteries and veins, and was the largest in the femoral veins, indicating that the veins are more sensitive to noradrenaline.

The antagonistic effects of SGB-1534, prazosin, phentolamine and yohimbine on the noradrenaline-induced contraction were compared in the arteries and the veins.

SGB-1534: SGB-1534 caused a parallel shift to the right of the concentration-response curve to noradrenaline in the arteries, but not in the veins (Fig. 1). For the two arteries, the plot of log (concentration ratio−1) (CR−1) vs. the negative logarithm of

![Fig. 1. Cumulative concentration-response curves to exogenous noradrenaline in mesenteric arteries (upper, left), mesenteric veins (lower, left) femoral arteries (upper, right) and femoral veins (lower, right) investigated in the absence (control) or presence of increasing concentrations of SGB-1534. Arterial and venous preparations, obtained from the same dogs, were used in the presence of 10^{-5} M cocaine and 10^{-6} M propranolol. Values are expressed as a percent of the maximal response to noradrenaline (see Table 1) and are shown as the means±S.E. of 5 preparations.](image-url)
Fig. 2. Cumulative concentration-response curves to exogenous noradrenaline in mesenteric arteries (upper, left), mesenteric veins (lower, left), femoral arteries (upper, right) and femoral veins (lower, right) investigated in the absence (control) or presence of increasing concentrations of prazosin (PZ). Arterial and venous preparations, obtained from the same dogs, were used in the presence of 10⁻⁵ M cocaine and 10⁻⁶ M propranolol. Values are expressed as a percent of the maximal response to noradrenaline (see Table 1) and are shown as the means±S.E. of 5 preparations.

Table 1. pD₂ values and maximum developed tension (MaxJT) in canine arteries and veins for noradrenaline

| Artery/Vein          | pD₂ value   | MaxJT (g)  |
|----------------------|-------------|------------|
| Mesenteric artery    | 5.92±0.06   | 35.6±2.1   |
| Femoral artery       | 5.79±0.05   | 14.8±0.8   |
| Mesenteric vein      | 6.17±0.03   | 6.0±0.8    |
| Femoral vein         | 6.38±0.05   | 4.1±0.8    |

Data represent means±S.E. of 20 preparations. There were significant (P<0.001) differences between the corresponding pD₂ values obtained from the artery and vein.

The molar concentration of SGB-1534 yielded a straight line with a negative slope significantly larger than 1.0 (upper part in Fig. 3, Table 2). The pA₂ value for SGB-1534 was slightly but significantly (P<0.05) smaller in the mesenteric artery than in the femoral one (Table 2). When compared on the pA₂ values of the four α-adrenoceptor antagonists in the arteries, the descending order of potency was: SGB-1534 > prazosin > phentolamine > yohimbine, indicating that among the four α-adrenoceptor antagonists SGB-1534 has the highest activity in blocking the noradrenaline-induced contraction.
Prazosin: Prazosin caused a parallel shift to the right of the concentration-response curve to noradrenaline in the arteries as well as in the veins (Fig. 4). In the arteries and the veins, the plot of log (CR-1) vs. the negative logarithm of the concentration of phentolamine was a straight line with a negative slope not significantly different from 1.0 (Table 2). The pA₂ value for phentolamine in the arteries was not significantly different from that in the veins.

Yohimbine: Yohimbine caused a parallel shift to the right of the concentration-response curve to noradrenaline in the four vessels (Fig. 5). The plot of log (CR-1) vs. the negative logarithm of the concentration of yohimbine was a straight line with a negative slope not significantly different from 1.0. The pA₂ value for yohimbine was significantly (P<0.001) larger in the veins than in the arteries (Table 2).

Discussion

The present experiment revealed that in the canine isolated femoral arteries and veins and in the mesenteric arteries and veins, exogenously applied noradrenaline concentration-dependently contracted these vessels; and the sensitivity to noradrenaline, when compared on pD₂ values, was significantly higher in the veins than in the arteries. The noradrenaline-induced contraction was abolished by the four α-adrenoceptor antagonists: SGB-1534, prazosin, phentolamine and yohimbine. It was noted that SGB-1534 and prazosin caused a parallel shift to the right of the concentration-response curve to noradrenaline in the arteries, but not in the veins. In the veins, unlike the arteries, SGB-1534 as well as prazosin caused a nonparallel depression of the concentration-response curve to noradrenaline. A similar phenomenon concerning prazosin was observed by De May and Vanhoutte (18) using the isolated femoral and splenic arteries and the femoral and saphenous veins of the dog.

According to Langer et al. (8), the α-adrenoceptors located in vascular tissues are classified into two subtypes, α₁- and α₂-adrenoceptors, determined using specific blockers for each receptor: Noradrenaline is an agonist at both α₁- and α₂-adrenoceptor subtypes. Prazosin is a selective α₁-adrenoceptor antagonist, and yohimbine is a...
Fig. 4. Cumulative concentration-response curves to exogenous noradrenaline in mesenteric arteries (upper, left), mesenteric veins (lower, left), femoral arteries (upper, right) and femoral veins (lower, right) investigated in the absence (control) or presence of increasing concentrations of phentolamine (PA). Arterial and venous preparations, obtained from the same dogs, were used in the presence of 10^{-5} M cocaine and 10^{-6} M propranolol. Values are expressed as a percent of the maximal response to noradrenaline (see Table 1) and are shown as the means±S.E. of 5 preparations.

relatively selective α_{2}-adrenoceptor antagonist. Phentolamine nonselectively blocks both α_{1}- and α_{2}-adrenoceptors. SGB-1534, like prazosin (8, 19), is a selective antagonist at α_{1}-adrenoceptors (10-14). De May and Vanhoutte (18) suggested the presence of mainly α_{1}-adrenoceptors in arteries and the presence of both α_{1}- and α_{2}-like postjunctional adrenoceptors in veins of the dog. Furthermore, Kou et al. (20) stated that the smooth muscle of the canine mesenteric vein possesses mainly the α_{2}-adrenoceptor subtype. Taking all these findings into consideration, it is not surprising that in the present experiment, SGB-1534 as well as prazosin caused a nonparallel shift to the right of the concentration-response curve to noradrenaline in the canine venous smooth muscles, since the presence of two subtypes of α-adrenoceptors was predicted particularly in the venous smooth muscle.

According to the present study, the pA_{2} values of phentolamine, in the mesenteric and femoral veins, were comparable to those obtained in the arteries, whereas those of yohimbine were significantly larger in the veins than in the arteries. This finding is consistent with the result obtained by De May and Vanhoutte (18). When the pA_{2} values of the four α-adrenoceptor antagonists in the arteries were compared, the descending order of potency was: SGB-1534>prazosin>phentolamine>yohimbine. Thus, SGB-1534, among the four α-adrenoceptor antagonists, had the highest potency in inhibiting the noradrenaline-induced contraction. On the other hand, it should be noted that contractions induced by the lower concentrations
of noradrenaline in the veins were not relatively blocked by SGB-1534 as well as prazosin. This phenomenon remains to be investigated. However, it would be possible to consider that in the veins, the lower concentrations of noradrenaline may produce mainly stimulation of $\alpha_2$-adrenoceptors, while the high concentrations primarily act upon $\alpha_1$-adrenoceptors.

In the present experiment, in the arteries, the plot of log (concentration ratio − 1) vs. the negative logarithm of the concentration of SGB-1534 was significantly larger than 1.0. Interestingly, such a phenomenon was not observed with prazosin: its slope was not significantly different from 1.0. Furthermore, our previous experiment (10) definitely demonstrated that in isolated guinea-pig aortae, SGB-1534, like prazosin, only caused a parallel shift to the right of the concentration-response curve to noradrenaline, and that the plot of log (concentration ratio − 1) vs. the negative logarithm of the concentration of SGB-1534 was on a straight line with a slope not significantly different from 1.0. It is difficult to explain why the slope of SGB-1534 was significantly different from 1.0 in the mesenteric and femoral arteries of the dog. Kenakin (21) stated that Schild regression with slopes greater than 1.0 can be produced by inadequate periods of equilibration for the tissue with the antagonist. Certainly, the potency of antagonists has been known to be dependent upon equilibration time. In view of this, the concentration-response curves for noradrenaline after preexposure to SGB-1534 for 60 min, instead of 10 min, were examined in a preliminary experiment. As a result, the
Table 2. Antagonistic effects of SGB-1534, prazosin, phentolamine and yohimbine on noradrenaline-induced contractions in canine arteries (A) and veins (V)

|                | r     | Slope           | pA₂ value          |
|----------------|-------|-----------------|--------------------|
|                |       | SGB-1534        |                    |
| Mesenteric A   | 0.93a | 1.53±0.05b      | 9.63±0.05c         |
| Femoral A      | 0.84a | 1.27±0.09b      | 9.61±0.09c         |
| Mesenteric V   | 0.83a | 1.12±0.16       |                    |
| Femoral V      | 0.77a | 1.38±0.22       |                    |
|                |       | Prazosin        |                    |
| Mesenteric A   | 0.90a | 1.04±0.04       | 8.32±0.10          |
| Femoral A      | 0.81a | 0.97±0.07       | 8.51±0.12          |
| Mesenteric V   | 0.70a | 0.96±0.17       |                    |
| Femoral V      | 0.94a | 1.11±0.11       |                    |
|                |       | Phentolamine    |                    |
| Mesenteric A   | 0.91a | 1.07±0.18       | 7.68±0.09          |
| Femoral A      | 0.84a | 1.00±0.10       | 7.70±0.09          |
| Mesenteric V   | 0.95a | 0.98±0.09       | 7.82±0.09          |
| Femoral V      | 0.93a | 1.02±0.12       | 7.80±0.17          |
|                |       | Yohimbine       |                    |
| Mesenteric A   | 0.96a | 0.96±0.05       | 7.08±0.09          |
| Femoral A      | 0.89a | 0.90±0.07       | 7.36±0.12          |
| Mesenteric V   | 0.97a | 0.94±0.04       | 7.62±0.03***       |
| Femoral V      | 0.98a | 1.00±0.03       | 7.76±0.05***       |

Slope values and regression coefficients (r) are obtained from the calculated Schild plots of log (concentration ratio - 1) against log molar (M) concentration of the antagonist. Data represent means±S.E. of 5 preparations. *: The correlation is statistically significant. b: The slope is significantly different from 1.0. c: As the slope of the Schild plot is significantly different from 1.0, these values should not strictly be regarded as pA₂ values. ***The difference from the pA₂ values obtained in the arteries is statistically significant (P<0.001).

In conclusion, the present results show that among the four α-adrenoceptor antagonists SGB-1534 possesses the highest activity in inhibiting the noradrenaline-induced contraction in the mesenteric and femoral arteries. Furthermore, contractile response to low concentrations of noradrenaline in the veins was not relatively inhibited by SGB-1534 and prazosin, indicating that the two selective α₁-adrenoceptor antagonists are less effective in the veins than in the arteries. These findings suggest that SGB-1534 as well as prazosin, as potent antihypertensive drugs, may exert more marked effects on arterial resistance vessels, through the α₁-adrenoceptor, leading to a pronounced hypotension.

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