Effects of Guanabenz on the Adrenergic Mechanism in Rabbit Arterial Strips

Yoshihiko Sakakibara, Ikunobu Muramatsu, Motohatsu Fujiiwara and Yasunori Nagasaka

Department of Pharmacology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

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Abstract—Effects of guanabenz, a hypotensive agent, on the adrenergic mechanism were studied in isolated rabbit thoracic aorta and pulmonary artery and findings were compared with data obtained with clonidine and guanethidine. Guanabenz in concentrations higher than $10^{-6}$ M produced weak contractions which were attenuated by phentolamine or yohimbine. Such concentrations of guanabenz competitively inhibited the contractile response to noradrenaline, but did not attenuate the response to tyramine. In concentrations ranging from $10^{-6}$ to $10^{-7}$ M, guanabenz attenuated the contraction and the increase of $^3$H-efflux in response to transmural electrical stimulation of the pulmonary artery preincubated with $^3$H-noradrenaline. Phentolamine or yohimbine effectively blocked these inhibitory effects of guanabenz. Such effects of guanabenz were similar to those of clonidine and dissimilar to those of guanethidine. These results indicate that guanabenz acts on presynaptic and postsynaptic alpha receptors of the peripheral blood vessels, as in the case of clonidine and that the potency was almost the same as clonidine.

Guanabenz (2,6-dichlorobenzylidene amidoguanidine acetate) is structurally composed of two distinct moieties of clonidine and guanethidine, respectively. This compound lowers the blood pressure and heart rate in experimental animals and humans (1-6). On the basis of in vivo pharmacological analysis, Baum et al. (2) and Baum and Shropshire (3) reported that hypotension may be induced by a decrease in sympathetic tone by the action of guanabenz on the sympathetic nerve endings and central sites. Bolm et al. (4) demonstrated that guanabenz reduced the turnover rate of noradrenaline and 5-hydroxytryptamine in the cat brain. From these pharmacological profiles, guanabenz has been considered as a "clonidine-like" centrally acting hypotensive drug (3, 7).

Clonidine produces at least three effects on the peripheral blood vessels; agonistic and antagonistic actions on the postsynaptic alpha receptor and agonistic action on the presynaptic alpha receptor (8). In this study, the effects of guanabenz on the rabbit peripheral arteries were examined and were compared with those of clonidine and guanethidine.

Materials and Methods

Rabbits of either sex, weighing 1.8 to 2.5 kg, were exsanguinated from the common carotid arteries and the thoracic aorta and the main pulmonary artery were isolated from the heart and lungs. For measurement of mechanical response, the thoracic aorta was
helically cut into strips which were approximately 3.0–4.0 mm in width and 15 mm in length, and then the strips were mounted vertically in an organ bath containing 20 ml of Krebs-Ringer’s solution of following composition (mM): NaCl 120.7; KCl 5.9; MgCl2 1.2; CaCl2 2.5; NaHCO3 15.5; NaH2PO4 1.2 and glucose 11.5. The bath medium was equilibrated with a gas mixture of 95% O2 and 5% CO2 before and during the experiment, and maintained at 37°C and pH 7.4. A resting tension of 1.5 g was applied and maintained during experiments. Each strip was equilibrated for 90 min before starting the experiments. During the equilibration period, the bath solution was changed every 20 min. Changes in muscle tension were recorded isometrically through a force-displacement transducer.

The pulmonary arteries were used to examine the effects on the response to transmural stimulation, as the thoracic aorta was less reactive to electrical stimulation. The pulmonary arteries were cut helically into strips, approximately 2 mm wide and 15 mm long, and they were set up in the same manner as described above. A resting tension of 1 g was applied. Electrical transmural stimulation was applied through a pair of platinum plates every 10 min. Stimulus parameters were 0.3 msec duration and frequencies 5, 10 and 30 Hz for 10 sec. Stimulus voltage was supramaximum.

In the study on release of 3H-noradrenaline, the main pulmonary arterial strips were preincubated with 3H-noradrenaline (3×10^-7 M) in Krebs solution containing ascorbic acid 100 mg/l for 90 min at 37°C. Thus, the arterial strips were placed between a pair of stimulating electrodes under 1 g of tension and superfused with the Krebs solution containing ascorbic acid at a flow rate of 1 ml/min (9, 10). The strips were equilibrated for at least 90 min before starting experiments. The stimulus parameters were the same as mentioned above. Superfusate samples were continuously collected every 1 min. Radioactivity in the superfusate was determined by counting in a Packard Tri-Carb liquid scintillation spectrometer after addition of 8 ml of scintillation fluid composed of 4 g PPO, 0.1 g POPOP and a 2:1 mixture of toluene and Triton X-100 to make 1,000 ml. The increase in 3H-efflux above the spontaneous efflux was calculated to be the net 3H-efflux evoked by electrical stimulation. The pA2 value was obtained according to the procedure of Arunlakshana and Schild (11). Statistical analysis was performed using the Student’s t-test for paired data.

The following drugs were used: L-[7, 8-3H] noradrenaline, specific activity 38.6 Ci/mmol (Amersham Buckinghamshire, England); L-noradrenaline bitartrate (Sigma, St. Louis, U.S.A.); guanabenz (WY-8678; Nippon-Shoji, Osaka, Japan); clonidine hydrochloride (Boeringer, Ingelheim, West Germany); guanethidine sulfate (Tokyo Kasei, Tokyo, Japan); yohimbine hydrochloride and tyramine hydrochloride (Nakarai, Kyoto, Japan); phentolamine mesylate (Regitine; Ciba, Basel, Switzerland).

RESULTS

Direct effects of guanabenz, clonidine and guanethidine: Guanabenz and clonidine caused slight but significant contractions in the rabbit aortic strips, and these responses were dose-related in concentrations ranging from 10^-6 to 10^-4 M. Guanethidine did not produce contraction. The dose-response curves of guanabenz and clonidine were significantly different from that of guanethidine as shown in Fig. 1. When these contractile responses were compared with those of noradrenaline, the amplitude of contraction induced by 10^-4 M guanabenz or clonidine was about 20% of the maximum contraction induced by 10^-5 M noradrenaline.
The pD₂ values of guanabenz, clonidine and noradrenaline were 5.08±0.01 (n=6), 5.58±0.03 (n=6) and 7.03±0.01 (n=6), respectively. The contractile responses by these three agents were markedly attenuated by 10⁻⁶ M phentolamine or 10⁻⁶ M yohimbine but not by 10⁻⁵ M guanethidine or 10⁻⁵ M bretylium.

Effects of guanabenz, clonidine and guanethidine on the contractile response to noradrenaline: Since guanabenz and clonidine exerted a weak but significant stimulating action on alpha receptors, the interaction with noradrenaline was examined. Figure 2A and 2B show the effects of guanabenz and clonidine on the contractile response to noradrenaline. Either agent in concentrations higher than 10⁻⁶ M shifted the dose-response curve of noradrenaline to the right. The slopes estimated by the method of Arunlakshana and Schild (11) were 1.01 and 1.16 for guanabenz and clonidine, respectively, indicating competitive inhibition. The pA₂ values for guanabenz and clonidine were calculated to be 6.47±0.06 (n=6) and 6.44±0.03 (n=6), respectively. On the other hand, guanethidine (10⁻⁶ M) shifted the dose-response curve of noradrenaline to the left (Fig. 2C).

Effects of guanabenz, clonidine and guanethidine on the tyramine-response: Tyramine (3X10⁻⁵ M) produced a contractile response in the rabbit aortic strips. The response was abolished by treatment with 3X10⁻⁵ M cocaine or 10⁻⁶ M phentolamine, thus suggesting that the contraction was elicited by noradrenaline released from the adrenergic nerve terminals. Guanabenz (10⁻⁷–10⁻⁶ M) and clonidine (10⁻⁷–10⁻⁶ M) did not affect or only slightly enhanced the contractile response to such a concentration of tyramine. However, guanethidine (10⁻⁶–10⁻⁵ M) markedly attenuated the tyramine-response, and the inhibition was not reversible by repeated washing (Fig. 3).

Effects of guanabenz, clonidine and guanethidine on electrical transmural stimulation in the pulmonary artery: When the pulmonary artery was electrically stimulated with the frequencies 5, 10 and 30 Hz, a frequency-dependent contractile response was elicited and this response was abolished by 10⁻⁷ M tetrodotoxin, 10⁻⁵ M bretylium or 10⁻⁶ M phentolamine, indicating that the contraction was sympathetic in origin. Guanabenz, clonidine and guanethidine inhibited the contractile response, and the inhibition was inversely related to stimulus frequency; the lower the frequency, the more evident was the inhibition (Figs. 4 and 5). The inhibition was also dependent upon concentrations, and guanabenz, clonidine or guanethidine in a low concen-
tration of $10^{-8}$ M showed a significant attenuation of the response to stimulation at 5 Hz. Yohimbine at $5 \times 10^{-9}$ M eliminated the inhibitory effects of $10^{-7}$ M guanabenz and clonidine on the response to electrical transmural stimulation at 5 and 10 Hz.

**Fig. 2.** Dose-response curve of noradrenaline in rabbit aorta in the presence of guanabenz (A), clonidine (B) and guanethidine (C). Noradrenaline was added cumulatively. The response induced by $10^{-5}$ M noradrenaline before treatment with each drug was taken as 100%. The blood vessels were treated with each concentration of drug 20 min before addition of noradrenaline. The values are the mean±S.E. of 6 experiments. Closed circles represent the response before treatment with drug. Open circles, triangles and squares are responses of each drug at concentrations of $10^{-7}$, $10^{-6}$ and $10^{-5}$ M, respectively.

**Fig. 3.** Representative recordings of the responses of the rabbit aorta to tyramine ($3 \times 10^{-5}$ M) (Ty) in the absence and presence of guanabenz and guanethidine. Tyramine was applied every 1 hr after washing. Preparations A and B were exposed to $10^{-7}$ M guanabenz and $10^{-6}$ M guanethidine, respectively, for 20 min before the second application of tyramine.
However, the inhibitory effect of $10^{-6}$ M guanethidine was not counteracted by $5 \times 10^{-8}$ M yohimbine (Fig. 4).

Tritium efflux induced by electrical transmural stimulation and effects of guanabenz, clonidine and guanethidine: In the pulmonary arteries preloaded with $^3$H-noradrenaline, electrical transmural stimulation produced a marked increase in $^3$H-efflux. The $^3$H-efflux evoked by transmural stimulation was abolished by $10^{-7}$ M tetrodotoxin or $10^{-5}$ M bretylium. Guanabenz, clonidine and guanethidine were added to the superfusion fluid 20 min prior to transmural stimulation. Addition of guanabenz ($10^{-8}$-$10^{-6}$ M) resulted in a significant inhibition of $^3$H-efflux without affecting the spontaneous efflux. The inhibition was more evident at lower frequencies and was dependent on concentrations. Clonidine ($10^{-8}$-$10^{-7}$ M) produced a similar inhibitory effect. As compared with the effects of $10^{-6}$ M guanabenz and clonidine, the inhibition due to $10^{-6}$ M guanethidine was more marked and nearly complete (Table 1).

Unlike with the effects of guanabenz or
Table 1. Effects of drugs on electrical transmural stimulation-induced $^3$H-efflux in rabbit pulmonary artery. The preparations were preincubated with $^3$H-noradrenaline, then superfused. The electrical transmural stimulation was applied at 30 min intervals.

| Drug         | Dose (M) | $^3$H-efflux (% of original)a |
|--------------|----------|-------------------------------|
|              |          | 5 Hz (n) | 10 Hz (n) | 30 Hz (n) |
| Guanabenz    | $10^{-8}$ | 48.7±2.7 (4) | 68.7±4.2 (4) | 89.1±1.8 (4) |
|              | $10^{-7}$ | 33.7±7.6 (4) | 60.1±8.7 (4) | 83.5±3.5 (4) |
|              | $10^{-6}$ | 31.9±4.6 (4) | 59.6±9.2 (4) | 75.2±3.7 (4) |
| Clonidine    | $10^{-9}$ | 59.0±4.3 (5) | 67.5±5.2 (4) | 81.0±5.1 (4) |
|              | $10^{-7}$ | 29.2±5.8 (4) | 55.9±6.5 (5) | 82.3±1.9 (4) |
|              | $10^{-6}$ | 17.6±3.0 (4) | 50.4±9.8 (5) | 81.7±2.2 (4) |
| Guanethidine | $10^{-8}$ | 9.4±2.5 (4)  | 2.4±0.2 (5)  | 4.2±0.3 (4)  |

All values obtained in the presence of the drugs were significantly different from corresponding control values (p<0.05). a: Stimulation-evoked $^3$H-efflux after treatment with drugs, expressed as percent of that obtained before treatment. n: Number of experiments.

clonidine, the arterial strips did not recover from the effects of guanethidine for at least 1 hour after washings with drug-free solution.

Figure 6 shows the effect of phentolamine on the guanabenz- and clonidine-induced reduction of $^3$H-efflux. The preparations were stimulated at 5 Hz. Phentolamine alone at $10^{-6}$ M augmented the increased $^3$H-efflux in response to transmural stimulation and suppressed the inhibitory effects of $10^{-8}$ M guanabenz and clonidine, but did not antagonize that of $10^{-8}$ M guanethidine.
DISCUSSION

Guanabenz, an antihypertensive agent, produced the following actions on isolated rabbit arteries: contraction, inhibition of the contractile response to noradrenaline as well as electrical transmural stimulation, and prevention of the release of noradrenaline. Similar effects were obtained with clonidine, and much different effects were seen with guanethidine.

The contractile response to guanabenz and clonidine was inhibited by phentolamine and yohimbine but not by guanethidine or bretylium, suggesting that the response was mediated through postsynaptic alpha receptors. However, the maximum efficacy and potency of these two agents were much lower than those of noradrenaline. In addition, guanabenz and clonidine competitively blocked the response to noradrenaline. Therefore, it is likely that guanabenz and clonidine have not only stimulating but also blocking actions on postsynaptic alpha receptors. The pressor and vasoconstrictor responses and the antagonism against noradrenaline of guanabenz in the dog or cat may be due to such dual actions (2).

The contractile response to electrical transmural stimulation was markedly attenuated by guanabenz, clonidine and guanethidine. These inhibitory effects of guanabenz and clonidine may also be in part explained by the alpha receptor blocking action mentioned above. However, the concentration required to inhibit the contractile response to electrical stimulation was 100 times lower than that required to inhibit the responses to exogenously applied noradrenaline response. Thus, the transmission failure cannot be explained only by postsynaptic alpha receptor blocking action.

Clonidine is known to stimulate presynaptic alpha receptors on the adrenergic nerve terminals and to reduce the transmitter release upon electrical stimulation (8, 12, 13). This feedback inhibition is more evident at lower frequencies of stimulation (14, 15). Clonidine effectively antagonizes response during low frequency nerve stimulation (7, 8, 13, 16). In the present study, guanabenz as well as clonidine markedly attenuated the stimulation-induced release of \(^3\)H-efflux from the adrenergic nerve terminals and the contractile response to electrical stimulation. These inhibitions were also selective at low frequencies. These results suggest that guanabenz, as well as clonidine, acts on presynaptic alpha receptors and inhibits the release of noradrenaline from the adrenergic nerve terminals upon electrical stimulation.

In fact, yohimbine, a selective presynaptic alpha receptor blocking agent (17, 18), eliminated the inhibitory effect of guanabenz on the contractile response to transmural stimulation, and phentolamine abolished the inhibitory effect of guanabenz on noradrenaline release, as was the case of clonidine. In contrast to these two agents, the inhibitory effect of guanethidine on the adrenergic transmission was not attenuated by the alpha receptor blocking agent.

The action of clonidine differs from that of guanethidine regarding the response to tyramine. Starke et al. (12, 19) have demonstrated that guanethidine irreversibly blocks response to tyramine, while clonidine has little effect. We found that guanabenz in low concentrations did not reduce the response to tyramine. This and the results mentioned above apparently indicate that guanabenz has “clonidine-like” actions on the peripheral arteries.

When the effects of guanabenz on the peripheral arteries are compared with those of clonidine, the potencies of the two drugs were found to be almost the same. In contrast, the dose of guanabenz required to produce a hypotension \textit{in vivo} is several times higher than clonidine (1). This different
effectiveness cannot be explained on the basis of the effects on the peripheral arteries; thus, this may reflect the difference of other mechanisms or processes such as penetration rate of the drugs to the central nervous system.

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