MODIFICATION BY AZ-55, GUANETHIDINE AND BRETYLIUM OF RESPONSES OF ATRIA AND AORTIC STRIPS TO TRANSMURAL STIMULATION

Noboru TODA, Hachiro USUI and Kiro SHIMAMOTO
Department of Pharmacology, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, and Biological Research Laboratories, Takeda Chemical Industries, Ltd., Higashiyodogawa-ku, Osaka

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A new antihypertensive drug (1), 1-cyclohexyl-3-guanidinoazetidine sulfate (AZ-55), structurally resembles guanethidine. It is widely known that guanethidine depresses peripheral adrenergic nerve function by preventing release of adrenergic nerve transmitters (reviewed by Boura and Green, 2). Bretylium also possesses the adrenergic neuron blocking action, but accumulated data point to the fact that bretylium and guanethidine fail to share all mechanisms of the blocking action (2).

The present study was aimed to investigate the effects of AZ-55 on responses of S-A nodes of the heart and of aortic smooth muscles to transmural neural stimulation, tyramine and noradrenaline. Effects were then compared with those of guanethidine and bretylium.

METHODS
Seventy-five albino rabbits of both sexes, weighing 1.8 to 2.2 kg, were used. Under ether anesthesia the animals were killed by bleeding from both common carotid arteries. The heart and the ascending and thoracic aortae were rapidly removed. Ventricles were discarded and conventional atrial preparation was prepared. The specimen was fixed horizontally between hooks under a resting tension of 300 to 450 mg in a 60 ml-capacity chamber containing the nutrient solution. Hooks anchoring the right atrial appendage were connected to the level arm of a force-displacement transducer (Nihonkoden Kogyo Co.). The bathing medium was maintained at 30±0.5°C and gassed with a mixture of 95% O₂ and 5% CO₂. The aorta was spirally cut into strips, which were vertically set under a resting tension of 2 g in a 50 ml-capacity bath. The bathing solution was maintained at 37±0.5°C.
and gassed with a mixture of 95% O2 and 5% CO2. Strips of the ascending aorta were used for studies on the contractile response to transmural electrical stimulation since the consistent, marked response could be obtained. For studies on the effect of noradrenaline, strips of the thoracic aorta were used. The composition of the nutrient solution was as follows (mM): Na+, 162.1; K+, 5.4; Ca++, 2.2; Cl−, 157.0; HCO3−, 14.9; dextrose, 5.6. After mounting the preparations, a 60- to 90 min equilibrium period for atria and a 120- to 150-min period for aortae were allowed before experimental procedures were begun.

A monopolar silver electrode, 0.5 mm in diameter and insulated to the tip, was used for transmural electrical stimulation of intracardiac cholinergic and adrenergic nerves innervating the S-A node (3). The transmural stimulation was the local application of square pulses, 0.1 msec in duration and about twice the threshold voltage of the nerve, applied at frequencies of 1, 5, 20 and 100/sec for a period of 3 sec. Spiral strips of the ascending aorta were placed between a pair of stimulating electrodes of platinum plate (5×15 mm, approx. 2 mm apart each other) as shown in an earlier report (4). The preparations were transmurally stimulated by a train of 0.3 msec-square pulses of supramaximal intensity (80 V by Toda et al., 5) applied at frequencies of 5, 20 and 100/sec for periods of 40, 10 and 2 sec, respectively. Thus, the total number of stimulus pulses was kept constant (200 pulses). Electrical pulses were delivered from an electronic stimulator (Nihonkoden Kogyo Co.).

Isometric contractions of the right atrium or the aortic strip were displayed on a two-channel penwriter (Sanei Sokki Co.). The S-A nodal rate was calculated from mean values of ten measurements of the cycle length between contractions. The cycle length was measured under steady state conditions and when the maximum response was attained following transmural stimulation, noradrenaline and tyramine. From vascular contractions induced by transmural stimulation two parameters were measured: the maximum tension developed and the duration of contraction at the level of half maximum tension, which will be termed 'duration' in this report. Values of the parameters obtained from preparations stimulated for 10 sec at a frequency of 20/sec in control media were taken as control (100%), and relative differences from the control were expressed as percent changes.

dl-Noradrenaline hydrochloride, tyramine hydrochloride, AZ-55 (1-cyclohexyl-3-guanidinoazetidine sulfate, Takeda Chemical Industries, Ltd.), guanethidine sulfate, bretylium tosylate and cocaine hydrochloride were used. Noradrenaline and tyramine were applied directly to the muscle bath in cumulative concentrations. The vascular tension developed at 5×10⁻⁶ M noradrenaline in control media was taken as 100%. Preparations were exposed to AZ-55, guanethidine and bretylium for 20 min and to cocaine for 10 min after which transmural stimulation, noradrenaline and tyramine were applied.

The results were expressed as mean values ± standard errors of the means. Comparisons of results were made using the Student's t-test.

RESULTS

Atrial preparations

The atrial rate was altered by exposure to AZ-55 from 83±4.8 beats/min (N=11) to 86±5.1 beats/min at 10⁻⁶ M (N=11), to 89±4.5 beats/min at 5×10⁻⁴ M (N=9) and to
FIG. 1. Alterations by AZ-55 of the positive and the negative chronotropic response to transmural stimulation. Figures in parentheses indicate the number of preparations. Mean values of the atrial rate under steady state conditions in control and AZ-55-added solutions were presented in the text.

FIG. 2. Alterations by guanethidine and bretylium of the positive and the negative chronotropic response to transmural stimulation. Figures in parentheses indicate the number of preparations. Mean values of the atrial rate under steady state conditions in control media, at $2 \times 10^{-7}$ M guanethidine and at $10^{-6}$ M guanethidine were $90 \pm 5.3$ beats/min ($N=6$), $93 \pm 5.5$ beats/min ($N=6$) and $95 \pm 5.7$ beats/min ($N=6$), respectively, whereas those in control media, at $5 \times 10^{-6}$ M bretylium and at $2 \times 10^{-5}$ M bretylium were $88 \pm 7.8$ beats/min ($N=8$), $91 \pm 3.0$ beats/min ($N=8$) and $95 \pm 4.5$ beats/min ($N=8$), respectively.
Fig. 3. Effects of AZ-55 on the positive chronotropic response to tyramine.

94±8.7 beats/min at $2 \times 10^{-5}$ M (N=4). The induced change in the rate was statistically insignificant.

Transmural stimulation applied to the S-A node caused a frequency-dependent decrease in the atrial rate followed by the increase. The induced bradycardia was not significantly affected by AZ-55 in concentrations up to $5 \times 10^{-6}$ M. On the other hand, the positive chronotropic effect of the nerve stimulation was apparently reduced by AZ-55: increase in the rate at a frequency of 100/sec in the presence of $10^{-6}$ M AZ-55 and at frequencies of 5, 20 and 100/sec in the presence of $5 \times 10^{-6}$ M was significantly less (P<0.01) than that in control media. The results are shown in Fig. 1. The inhibitory effect of AZ-55 at $5 \times 10^{-6}$ M was prevented by prior application of $3 \times 10^{-6}$M cocaine in 3 atria, but the effect, once established, was not reversed by cocaine in 2 preparations.

Guanethidine in concentrations of $2 \times 10^{-7}$ and $10^{-6}$ M reduced the positive chronotropic effect of transmural stimulation in a dose-dependent manner (significant difference from control, P<0.01) (Fig. 2). Bretylium ($5 \times 10^{-5}$ and $2 \times 10^{-5}$ M) also elicited a significant inhibition of the positive effect of transmural stimulation (P<0.01) at 100/sec in the presence of $5 \times 10^{-6}$ M and at 5, 20 and 100/sec at $2 \times 10^{-5}$ M (Fig. 2). Both drugs preferentially suppressed the response to high stimulus frequency (100/sec). The negative chronotropic effect was not influenced.

The dose-chronotropic response curve of tyramine was moved right by AZ-55 in concentrations ranging from $2 \times 10^{-7}$ to $5 \times 10^{-6}$ M (Fig. 3). Guanethidine ($2 \times 10^{-7}$ and $10^{-6}$ M) also shifted the dose-response curve to the right, whereas bretylium ($5 \times 10^{-5}$ and $2 \times 10^{-5}$ M) shifted the curve to the left: increase in the rate at $6 \times 10^{-4}$ and $3 \times 10^{-2}$ M tyramine
in the presence of bretylium was significantly greater than that without bretylium (P<0.01) (Fig. 4). Repeated wash of preparations with control media reversed the bretylium action.

The positive chronotropic effect of noradrenaline was augmented by AZ-55 (at $5 \times 10^{-7}$

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**Fig. 4.** Effects of guanethidine and bretylium on the positive chronotropic response to tyramine. After wash: 60 to 90 min after repeated wash of preparations with fresh solutions.

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**Fig. 5.** Effects of AZ-55 on the positive chronotropic response to noradrenaline.
Fig. 6. Effects of guanethidine and bretylium on the positive chronotropic response to noradrenaline.

Fig. 7. Changes by AZ-55 in the contractile response of aortae to transmural stimulation. Abscissa: frequency and period of stimulation. Contractions induced by the stimulation at 20/sec for 10 sec in control media were taken as 100%: mean values of the tension developed and the duration of contraction were 0.94±0.13g and 1.18±0.08 min, respectively (N=9). Figures in parentheses indicate the number of preparations.
M noradrenaline, significant difference between values at 0 and $5 \times 10^{-6} \text{ M AZ-55, } P<0.02$) (Fig. 5). Similar augmentation of the effect of noradrenaline was produced by bretylium. Trends of augmenting the chronotropic effect was also observed following treatment with guanethidine. Results are illustrated in Fig. 6.

**Aortic preparations**

Transmural stimulation applied to strips of the ascending aorta elicited a transient increase in the tension. AZ-55 at $10^{-8}$ and $5 \times 10^{-6} \text{ M}$ significantly inhibited the tension developed by stimulation at frequencies of 5, 20 and 100/sec and caused a roughly parallel shift of the frequency-response curve downwards (Fig. 7). The duration of contractions was shortened in association with a decrease in the developed tension. The resting tension was not appreciably influenced by AZ-55 in concentrations up to $5 \times 10^{-6} \text{ M}$.

Contractile response to transmural stimulation was also significantly inhibited by guanethidine ($2 \times 10^{-7}$ and $10^{-6} \text{ M}$) and bretylium ($5 \times 10^{-6}$ and $2 \times 10^{-5} \text{ M}$) (Fig. 8).

In 2 preparations cocaine at $3 \times 10^{-6} \text{ M}$ increased the contractile response to transmural stimulation by 26 and 51%, and prevented the inhibitory effect of AZ-55 ($5 \times 10^{-6} \text{ M}$), however, cocaine failed to restore the response to the nerve stimulation in 5 of 5 preparations in which the inhibition had already been established. On the other hand, following cocaine the inhibition by guanethidine ($10^{-6} \text{ M}$) was antagonized completely in one and partially (about 50%) in another one of 5 preparations but was not reversed in the remaining 3.

![Fig. 8. Changes by guanethidine and bretylium in the contractile response of aortae to transmural stimulation. Contractions induced by the stimulation at 20/sec for 10 sec in control media were taken as 100% : mean values of the tension developed and the duration of contraction were 1.00±0.15g and 1.34±0.13 min (N=9), respectively, for experiments with guanethidine, and those were 0.72±0.13g and 1.34±0.13 min (N=8), respectively, for experiments with bretylium.](image-url)
FIG. 9. Effects of cocaine in aortic preparations in which the inhibitory effect of AZ-55 and guanethidine on the response to transmural stimulation was established. Ordinate: percent change in the tension developed, relative to that developed by stimulation at 20/sec for 10 sec in control media (100%). No significant difference is observed in the figure.

FIG. 10. Dose-response curves of noradrenaline in aortic strips in the presence or absence of AZ-55. Tension developed at $5 \times 10^{-7}$ M noradrenaline in control media was taken as 100%: mean value of the tension was 3.26 ± 0.26 g (N = 10).

FIG. 11. Dose-response curves of noradrenaline in aortic strips in the presence or absence of guanethidine and bretylium. Tension developed at $5 \times 10^{-7}$ M noradrenaline in control media was taken as 100%: mean values of the tension were 4.22 ± 0.33 g (N = 8) for experiments with guanethidine and 3.43 ± 0.29 g (N = 8) for experiments with bretylium.
values of contractions induced by transmural stimulation in the presence of AZ-55 and guanethidine and those in combination with cocaine are shown in Fig. 9.

Contractile effect of noradrenaline was increased by treatment with AZ-55 in concentrations of $10^{-6}$ and $5 \times 10^{-6}$ M (in concentrations of noradrenaline higher than $2.5 \times 10^{-6}$ M, significant difference from control, $P<0.01$) (Fig. 10). The amine effect was also increased by guanethidine (at $10^{-5}$ and $5 \times 10^{-5}$ M noradrenaline, significant difference from control, $P<0.01$) and bretylium (significant difference from control, $P<0.01$, at all but the lowest concentration of noradrenaline) (Fig. 11).

**DISCUSSION**

A new azetidine derivative, 1-cyclohexyl-3-guanidinoazetidine (AZ-55), caused a dose-dependent inhibition of responses of the S-A nodal pacemaker and the aortic smooth muscle to transmural neural stimulation as did guanethidine and bretylium. The dose of AZ-55 to cause a 50% inhibition of the responses of atria and aortae stimulated at 20/sec was approx. the same (between $10^{-6}$ and $5 \times 10^{-6}$ M). In atrial preparations AZ-55 is approx. 1/10 times as potent as guanethidine for inducing the 50% inhibition, and about 5 times as potent as bretylium, while in aortic strips AZ-55 is approx. 1/5 times as potent as guanethidine and 2 to 3 times as potent as bretylium. It is shown that in nictitating membranes (6, 7, 8), femoral vessels and spleens of the cat (9), in perfused ear vessels of the rabbit (9) and in isolated vas deferens of the guinea pig (10) bretylium causes relatively greater inhibition of responses to high stimulus frequencies and depresses the slope of frequency-response curves, whereas guanethidine preferentially suppresses responses to low frequencies and causes roughly parallel shifts of the curves. The results obtained here from isolated rabbit aortae are consistent with the aforementioned. AZ-55, like guanethidine, caused parallel shifts of the frequency-response curves. On the other hand, no qualitative difference between AZ-55, guanethidine and bretylium was detected in the present study on the chronotropic response to transmural stimulation: the inhibitory effect of these drugs at a stimulus frequency of 100/sec was greater than that at the lower frequencies. No difference between guanethidine and bretylium is also observed in studies of the inhibitory response of isolated rabbit ileum to periarterial nerve stimulation (9).

It is demonstrated that desipramine prevents the pharmacologic effects of guanethidine in experimental animals (11, 12). The present study proved that cocaine, a known inhibitor of the amine uptake, prevented the inhibitory effects of guanethidine and AZ-55 on the responses of atria and aortae to transmural stimulation but did not reverse those, once established. Cocaine, like desipramine, would inhibit uptake of guanethidine and AZ-55 and thereby prevent the pharmacologic effects of the adrenergic neuron blocking agents (13) but fails to extrude the agents accumulated intraneuronally (14). On the other hand, a bretylium-induced inhibition in the atrial and aortic responses, even though it is established, is completely reversed by cocaine (unpublished data).

Vernikos-Danellis and Zaimis (15) observed that bradycardia caused by peripheral vagal stimulation in anesthetized cats was increased by guanethidine and bretylium, and suggested
that this was attributed to blockade of the cardiac adrenergic innervation. In contrast, a concentration of bretylium sufficient to abolish the response of isolated rabbit atria to sympathetic stimulation was found to depress the response to stimulation of preganglionic vagus nerves (16). In the present experiments in which postganglionic cholinergic and adrenergic nerves in the heart were simultaneously stimulated (3, 17), AZ-55 as well as guanethidine and bretylium did not significantly influence the cholinergic response of the S-A node to transmural stimulation.

The positive chronotropic effect of tyramine was inhibited by AZ-55 and guanethidine in concentrations sufficient to block the positive response to transmural stimulation, but was significantly augmented by bretylium. The effect of noradrenaline was augmented by these three. Benfey and Greef (18) and Kadzielawa (19) observed that guanethidine inhibited the action of tyramine and potentiated the effect of noradrenaline on isolated guinea pig and rabbit atria. According to Bhagat and Shideman (20), treatment of rats for 2 hours with guanethidine decreases the concentration of cardiac catecholamines and inhibits the uptake of exogenous noradrenaline, while treatment with bretylium shows no effect on either. Iversen (21) showed the inhibitory effect of these two on the uptake of noradrenaline by the isolated perfused heart, although the ID₅₀ of guanethidine was 1/5 that of bretylium. It appears that guanethidine, and possibly AZ-55, inhibits the uptake of tyramine by the heart and that bretylium fails to interfere with the uptake of tyramine and also the release of noradrenaline from stored sites, thus permitting the amine to affect sensitized receptive sites (15, 22). In the present study, however, no evidence showing inhibition of the amine uptake was obtained in aortae exposed to AZ-55, guanethidine and bretylium. Cocaine and desipramine are demonstrated to prolong markedly the duration of aortic contraction, which is thought to derive from the inhibition of the amine uptake (4).

It is concluded that AZ-55 causes selective blockade of the release of adrenergic nerve transmitters in atria and aortae as does guanethidine and bretylium. It appears that the mechanism of action of AZ-55 resembles that of guanethidine but is largely different from that of bretylium.

**SUMMARY**

1. Effects of AZ-55 (1-cyclohexyl-3-guanidinoazetidine sulfate), guanethidine and bretylium on the response of atria and spiral strips of the ascending aorta from rabbits to transmural electrical stimulation were investigated.

2. AZ-55 (10⁻⁶ and 5×10⁻⁷ M) caused a dose-dependent inhibition of the positive chronotropic response and the contractile response of aortae to transmural stimulation, as did guanethidine (2×10⁻⁷ and 10⁻⁶ M) and bretylium (5×10⁻⁶ and 2×10⁻⁵ M). The negative chronotropic effect of transmural stimulation was not influenced by these three drugs.

3. The inhibitory effect of AZ-55 and guanethidine on the atrial and vascular response to transmural stimulation was prevented by prior application of cocaine (3×10⁻⁶ M), however, cocaine failed to reverse the action of AZ-55 and guanethidine, once established.
4. The dose-chronotropic response curve of tyramine was moved right by AZ-55 and guanethidine but was moved left by bretylium. In both the atrial and vascular preparations the three drugs shifted the dose-response curve of noradrenaline to the left.

5. The findings on AZ-55 are discussed in relation to guanethidine and bretylium which showed different properties of the adrenergic neuron blockade.

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