Angiotensin AT₁–Receptor Blockers Enhance Cardiac Responses to Parasympathetic Nerve Stimulation via Presynaptic AT₁ Receptors in Pithed Rats

Fumiko Yamaki¹*, Takanori Arai², Masato Aoyama¹, Akane Watanabe¹, and Yoshinobu Takata¹

¹Department of Pharmacology, Ohu University School of Pharmaceutical Sciences, 31-1 Misumido, Tomita-machi, Koriyama, Fukushima 963-8611, Japan
²Department of Pharmacology, Faculty of Pharmaceutical Sciences, Teikyo University, 2-11-1 Kaga, Itabashi, Tokyo 173-8605, Japan

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Abstract. In the present study, we investigated the effects of angiotensin AT₁–receptor blockers, KT3-671 and losartan, on the cardiac vagal neurotransmission in pithed rats. The bradycardia induced by vagal nerve stimulation (VNS, at 5 Hz) was potentiated significantly and dose-dependently by KT3-671 and also losartan. This enhancement effect of KT3-671 (10 mg/kg) was slightly potent than that of losartan (10 mg/kg). On the other hand, an angiotensin AT₂–receptor blocker, PD123319 (10 mg/kg), did not affect VNS-induced bradycardia. KT3-671 and losartan did not affect the exogenous acetylcholine-evoked bradycardia. Intravenous infusion of AngII (100 ng/kg per min) attenuated the VNS-induced bradycardia. This inhibitory effect of AngII on bradycardia was restored by both KT3-671 and losartan. These results suggest that endogenous AngII can have a tonic inhibitory effect on cardiac vagal transmission by stimulating the presynaptic AT₁ receptors not AT₂ receptors. Suppression of this mechanism by the AT₁-receptor blockers causes the facilitation of acetylcholine release from vagal nerve endings. This acceleratory effect of AT₁-receptor blockers in cardiac vagal neurotransmission may contribute to the lack of reflex tachycardia following hypotension.

Keywords: angiotensin AT₁–receptor blocker, angiotensin II, vagal neurotransmission, bradycardia, rat heart

Introduction

Angiotensin II (AngII) plays an important role in the regulation of systemic blood pressure. Therefore, anti-angiotensin drugs such as angiotensin-converting enzyme inhibitors (ACEI) and angiotensin AT₁–receptor blockers (ARB) are useful for the treatment of hypertension. ACEI and ARB decrease blood pressure without producing reflex tachycardia in humans (1 – 6). Reflex tachycardia as a consequence of hypotension results from both increased sympathetic activity and decreased vagal one, but there are some reports suggesting that ACEI (7 – 9) and ARB (10, 11) appear to have no effect on the tachycardia due to cardiac sympathetic nerve stimulation in pithed rats.

On the other hand, an ACEI, captopril, potentiated the vagal bradycardia but the bradycardia by the muscarinic M₂ receptors activation was not enhanced in pithed rats (8, 9). In addition, Potter et al. demonstrated that exogenous AngII inhibited the bradycardia induced by vagal nerve stimulation but not by exogenously administered acetylcholine (ACh) (12). These observations suggest that not only endogenous but also exogenous AngII suppressed vagal nerve neurotransmission and this inhibitory effect of AngII on vagal bradycardia is attributed to the inhibition of ACh release from the vagal nerve terminals. In fact, Kawada et al. demonstrated that AngII suppressed ACh release through AT₁ receptors.
located on parasympathetic ganglia in the in vivo cat heart (13). In contrast, there are some reports demonstrating that AngII did not inhibit the bradycardia induced by vagal stimulation in the anesthetized ferret (14) and in the pithed rat (8). At present, the differences in these observations are not resolved.

KT3-671, a non-peptide ARB with no active metabolites (15), induced hypotension without causing reflex tachycardia in various animal models of hypertension (15 – 17). Takata et al. showed that neither KT3-671 nor losartan affected cardiac sympathetic neurotransmission in pithed rats (11). On the other hand, they showed that both ARB inhibited vascular sympathetic neurotransmission, and this vascular sympathoinhibitory effect by KT3-671 was more potent than that by losartan.

In the present study, we investigated the effects of KT3-671, losartan, and exogenous AngII on the vagal transmission in pithed rats in order to examine the mechanism responsible for the lack of reflex tachycardia. The effects of KT3-671 were also compared with those of losartan.

Materials and Methods

This study was conducted in accordance with the guidelines for the Care and Use of Laboratory Animals of Ohu University School of Pharmaceutical Sciences and the guidelines of the Animal Care and Use Committee of Teikyo University.

Pithed rat preparation and general procedures

Male Wistar rats (300 – 450 g; Nihon SLC, Inc., Shizuoka) were anesthetized with diethyl ether. The left carotid artery and both femoral veins were cannulated to measure blood pressure and to administer drugs, respectively. Vagal nerves were bilaterally cut at the cervical region. After tracheal cannulation, the rats were artificially ventilated with room air using a rodent respirator at a rate of 70 breaths/min with a volume of 1 ml/100 g body weight. An appropriate volume of a gas mixture of 95% O₂ and 5% CO₂ was additionally supplied through a tracheal cannula to keep arterial blood gases within the physiological range. Under reanesthesia with sodium thiopental (20 mg/kg, i.v.), the animals were placed in a stereotactic headholder and the cervical cord was transected at the C1 level. Cardiovascular responses were obtained by stimulating the peripheral end of the left vagal nerve through bipolar platinum electrodes with an electric stimulator (SEN-7103; Nihon Kohden, Tokyo). Stimulation parameters to produce bradycardia and hypotension were 5 Hz, 0.2 ms, supramaximal voltage (30 V) for 30 s, unless otherwise indicated. In our previous report, we confirmed that the bradycardic and hypotensive responses to electrical stimulation were abolished by tetrodotoxin (8 μg/kg, i.v.) and by atropine (3 mg/kg, i.v.) (9). Heart rate and blood pressure were measured by a heart rate counter (AT-600G, Nihon Kohden) triggered by blood pressure waves and by a pressure transducer (TP-200TL, Nihon Kohden), respectively, and the two parameters were simultaneously captured on a recorder (RJG-4124, Nihon Kohden). After surgery, the anesthetic was switched to urethane + α-chlorarose (0.6 g/kg + 0.06 g/kg, i.p.). Rectal temperature was maintained 37°C – 38°C by a heating lamp. All rats were allowed 30 – 60 min to recover from preparatory surgery for stabilization of heart rate and blood pressure. All rats received tubocurarine (1 mg/kg, i.v.) to prevent muscle movement during electric stimulation and propranolol (1 mg/kg, i.v.) to exclude β-adrenoceptor-mediated effects. Continuous infusion of AngII and ACh was carried out using the peristaltic pump (PST-110; Iwaki, Tokyo).

Drugs

KT3-671 was supplied by Kotobuki Pharmaceutical (Nagano). Urethanes, α-chlorarose, AngII, and PD123319 di (trifluoroacetate) salt hydrate were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Losartan potassium, diethyl ether, d-tubocurarine were obtained from Wako Pure Chemical Industries (Osaka). Sodium thiopental was purchased from Mitsubishi Tanabe Pharmaceutical Corporation (Osaka). Propranolol hydrochloride was purchased from AstraZeneca K.K. (Osaka), and acetylcholine chloride was purchased from Daiichi Sankyo Company (Tokyo).

Statistical analyses

Results are expressed as means ± S.E.M. Statistical analyses were performed using a paired t-test or an unpaired Student’s t-test for two-sample comparison and ANOVA followed by Dunnett’s test for multiple comparisons. A P-value less than 0.05 were considered significant.

Results

Effects of ARB and PD123319 on bradycardiac responses to VNS

In pithed rats, vagal nerve stimulation (VNS) caused bradycardia of about 44 ± 1 beats/min (n = 55). KT3-671 (5 and 10 mg/kg, Fig. 1A) significantly enhanced the VNS-induced bradycardia over the next nine stimuli in a dose-dependent manner. This enhanced effect of KT3-671 (10 mg/kg) was 124% – 130% in comparison with the control. Losartan (5 and 10 mg/kg, Fig. 1B) also significantly potentiated the bradycardia induced
Losartan (10 mg/kg) augmented the VNS-induced bradycardia by 118% – 120% as compared with the control. On the other hand, PD123319 (10 mg/kg), a selective angiotensin AT2–receptor blocker, did not affect the VNS-induced bradycardia (Fig. 1C). KT3-671, losartan, and PD123319 caused no significant change in basal heart rate (data not shown).

Effects of AngII infusion and subsequent ARB on the VNS-induced bradycardia

As shown in Fig. 2, continuous infusion of AngII (100 ng/kg per min) significantly inhibited the VNS-induced bradycardia by 20% – 30% as compared with the control value. This inhibitory effect of AngII was sustained for at least 34 min after initiation of infusion. The subsequent administration of KT3-671 (10 mg/kg) or losartan (10 mg/kg) significantly reversed the VNS-induced bradycardia further than pretreatment of AngII. These reversal effects of KT3-671 and losartan were 109% – 112% and 101% – 109% in comparison with the control, respectively. AngII infusion did not influence basal heart rate (data not shown).

Effects of ARB and AngII infusion on the ACh-induced bradycardia

The infused dose (120 – 480 µg/kg per min) of ACh was adjusted in each experiment to obtain a similar bradycardia to that induced by VNS in pithed rats. ACh caused bradycardia of about 42 ± 2 beats/min (n = 20). As shown in Fig. 3, none of KT3-671 (10 mg/kg), losartan (10 mg/kg), and AngII (100 ng/kg per min) affected the ACh-induced bradycardia.

Discussion

In the present study with pithed rats, KT3-671 as well as losartan significantly enhanced the VNS-induced bradycardia in a dose-dependent manner. In contrast, neither KT3-671 nor losartan potentiated the bradycardia.
caused by infusion of ACh. Furthermore, PD123319, a selective angiotensin AT1 receptor blocker, did not influence vagal bradycardia. These results reflect that both KT3-671 and losartan augment the ACh release from the vagal nerve terminals via blockade of the presynaptic AT1 receptors, not AT2 receptors. In other words, endogenous AngII persistently suppresses the ACh release through activation of the presynaptic AT1 receptors. In the present study, we demonstrated that infusion of AngII (100 ng/kg per min, i.v.) inhibited the VNS-induced bradycardia but not the ACh-induced one. Furthermore, the subsequent administration of KT3-671 or losartan reversed this inhibitory effect of AngII and showed further bradycardia.

It is widely known that ACEI and ARB induce hypotension without causing reflex tachycardia in humans (1 – 6). In various hypertension models, KT3-671-induced blood pressure reduction also is not accompanied by a reflex tachycardia (15 – 17). It has been reported that KT3-671 as well as losartan inhibited vascular but not cardiac sympathetic neurotransmission in pithed rats (11). In that paper, it showed that KT3-671 was approximately four times more potent than losartan in inhibiting vascular sympathetic neurotransmission. In the present study, we demonstrated that VNS-induced bradycardia was significantly and dose-dependently potentiated by KT3-671 and also losartan. In addition, KT3-671 (10 mg/kg) enhanced VNS-induced bradycardia by 124% – 130% over the next nine stimuli, while losartan (10 mg/kg) potentiated vagal bradycardia by 118% – 120%. Although not reaching a statistically significant difference, KT3-671 seems to be slightly more potent than losartan in enhancing cardiac vagal neurotransmission. These data indicate that the enhancement effect of cardiac vagal neurotransmission by both ARB participates in the absence of reflex tachycardia, and KT3-671 induces hypotension more effectively and without causing reflex tachycardia in comparison with losartan.

Previously, Rechtman et al. (8) also had shown that losartan enhanced vagal bradycardia in pithed rats. In that paper, however, they examined the effect of losartan only at one dose (10 mg/kg). Additionally, they showed that infusion of AngII (30 ng/kg per min, i.v.) did not inhibit vagal bradycardia. As a reason for the absence of the inhibitory effect of AngII, they described that endogenous AngII already maximally activated AngII receptors in pithed rats. Andrews et al. (14) also demonstrated that a high dose (500 ng/kg or 2 µg/kg, i.v.) of AngII did not show any effect on the cardiac or gastric responses to vagal nerve electrical stimulation in anesthetized ferrets.

We showed that losartan enhanced vagal bradycardia dose-dependently and that 5 mg/kg of losartan also
significantly potentiated VNS-induced bradycardia. In addition, intravenous infusion of AngII (100 ng/kg per min) significantly suppressed VNS-induced bradycardia, and the subsequent administration of ARB reversed this inhibitory effect of AngII. As well as our data, Potter et al. (12) reported that a bolus injection of AngII (5 – 10 µg, i.v.) almost completely abolished vagal bradycardia in anesthetized dogs. This study also showed that the addition of AngII (2 – 5 µg / 25 ml) to the organ bath reduced the bradycardia induced by electrical stimulation of the vagal nerve in the isolated atria from guinea pigs.

In pithed rats, plasma renin activity (18) and AngII concentration (19) are higher compared with normal rats. However, in addition to our findings that AngII inhibited vagal bradycardia, Takata et al. also demonstrated that infusion of angiotensin I (200 ng/kg per min) significantly suppressed the VNS-evoked bradycardia in pithed rats (9). If endogenous AngII already reaches a maximal level in pithed rats, infusion of AngII or angiotensin I could not exhibit an additional effect. This discrepancy might be related to differences in dose of AngII, experimental animals, anesthesia, and stimulation condition of the vagal nerve.

There is a report suggesting that not only AT1 receptors but also AT2 receptors participate in AngII-mediated facilitation of norepinephrine release in human atria (20). However, we demonstrated that PD123319 (10 mg/kg) did not affect vagal bradycardia, which suggests that presynaptic AT2 receptors do not exist in cardiac parasympathetic nerve terminals and/or not contribute to ACh release from vagal nerve terminals in pithed rats. Our data with pithed rats indicate that not only endogenous AngII but also exogenous AngII can suppress VNS-induced bradycardia. Furthermore, KT3-671 and losartan seem to enhance vagal nerve activity by canceling this inhibitory effect of endogenous AngII at AT1 receptors of vagal nerve endings.

It has been reported by Kawada et al. that intravenous AngII (10 µg/kg per hour) attenuated myocardial interstitial ACh release in response to vagal nerve stimulation in anesthetized cats (13). Moreover, they showed that this inhibitory effect of AngII on vagal stimulation-induced ACh release was reduced by intravenous pretreatment of losartan (10 mg/kg) but not by local administration of losartan (10 mM) through a dialysis probe implanted in the wall of the left ventricle. These findings indicate that AngII suppresses ACh release through activation of AT1 receptors located on parasympathetic ganglia. We did not investigate the inhibitory action site of AngII in the present study, but the possibility that AngII may inhibit neurotransmission by acting on the parasympathetic ganglia cannot be excluded.

In conclusion, both KT3-671 and losartan enhanced the bradycardia induced by vagal nerve stimulation in pithed rats. AngII inhibited vagal bradycardia through activation of presynaptic AT1 receptors, suggesting that the two ARB enhanced cardiac vagal neurotransmission via excluding this inhibitory effect of AngII. The findings of the present study provide one reason why ARB are devoid of the reflex tachycardia. In comparison with losartan, KT3-671 seems to be more potent in inhibition of vascular sympathetic neurotransmission and enhancement of cardiac vagal neurotransmission.

References