Full Paper

The Preferential Inhibitory Effect of Olmesartan, a New Angiotensin II Type 1 Antagonist, on Sympathetic Nerve Terminals in Isolated Canine Splenic Artery

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Abstract. Effects of olmesartan (RNH-6270: (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl[4-[2-(tetrazol-5-yl)-phenyl]phenyl]methylimidazol-5-carboxylase, an active form of olmesartan medoxomil (CS-866)) was investigated in isolated, perfused canine splenic arterial preparations. Neither exogenous noradrenaline- nor ATP-induced vasoconstrictor responses were modified by treatment with the used concentrations of olmesartan (1 – 100 nM). A high concentration of 10 nM angiotensin II caused a potentiation of either noradrenaline- and ATP-induced constrictions, although 1 nM angiotensin II did not induce any potentiating effects for these responses. These potentions were inhibited by olmesartan in a concentration-related manner. Periarterial nerve electrical stimulation (PNS) readily induced a biphasic constriction consisting of an initial P2X purinoceptor-mediated vasocostriction followed by a prolonged mainly α₁-adrenoceptor-mediated response. PNS-induced 1st and 2nd peaked responses were significantly inhibited by olmesartan in a concentration-related manner. With a low concentration of 1 nM angiotensin II, which did not induce any vascular effects by itself, PNS-induced responses were markedly enhanced. The enhanced responses were inhibited by olmesartan. It is concluded that endogenous angiotensin II exerts its stimulating action on the releases of ATP and noradrenaline from the periarterial sympathetic nerve terminal, and olmesartan has an inhibitory property on angiotensin II-induced potentiation of endogenous ATP- and noradrenaline-induced responses.

Keywords: angiotensin AT₁ receptor, isolated canine splenic artery, periarterial nerve electrical stimulation, angiotensin II

Introduction

In 1995, the pharmacological profiles of CS-866 (olmesartan medoxomil), a novel nonpeptide angiotensin receptor antagonist were reported; i.e., orally administered CS-866 produced a long-lasting inhibition of angiotensin II-induced pressor responses (1). It was also reported that olmesartan has more potent inhibitory action against angiotensin II-induced contraction and angiotensin II-induced pressor response than losartan and EXP3174 in isolated guinea pig aorta and in conscious rats (1). Since SK&F-525A (a P-450 inhibitor) suppressed the angiotensin II inhibitory effect of losartan but not that of CS-866, this drug might be not affected by drug metabolizing enzymes in the liver. Thus, CS-866 is expected to be potential candidate as one of the antihypertensive drugs that are effective on a once-daily dose regimen. However, the pharmacological characteristics of CS-866 have not been precisely investigated yet.

It has been well recognized that angiotensin II causes a facilitation of noradrenaline release from sympathetic nerve terminals (2). In 2001, it was reported that angiotensin II increased releases of a neurotransmitter (ATP) to mimic the effects of raising extracellular Ca²⁺ concentration in the guinea pig vas deferens (3). In 1998, it was observed that the time course of vasoconstrictor responses to pulse trains of up to 30-s duration of electrical stimulation of periarterial sympathetic nerve fibers...
(PNS) appeared to be biphasic vasoconstrictions consisting of an initial purinergic phase followed by a predominant adrenergic phase (4, 5).

In the present study, we attempted to clarify the effects of olmesartan (RNH-6270), an active form of CS-866, on biphasic vasconstrictor responses to PNS and exogenously administered noradrenaline- and ATP-induced responses in isolated, perfused canine splenic arterial preparations, which were developed by Hongo and Chiba (6) and modified by Tsuji and Chiba (7).

Materials and Methods

Arterial preparations

Mongrel dogs of either sex, weighing 8 – 15 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), the dogs were killed by rapid exsanguinations from the right femoral artery. The arterial main branches of the splenic artery were isolated and side branches of the artery were tied with silk threads. Then, the artery (1 – 1.2 mm in an outer diameter) was cut into segments (15 – 20 mm in length). Four segments were obtained from each splenic artery. Each segment was cannulated and set up for perfusion as described previously (6 – 8). Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. A proximal portion of the segment was fixed to the distal arterial segment from the distal to the proximal end. A cannula was 3 – 4 cm long and 0.8 – 1.0 mm in outer diameter. The proximal portion of the needle-type cannula with silk threads. The cannula was 3 – 4 cm long and 0.8 – 1.0 mm in outer diameter with small side holes 5 mm from the distal sealed end. The cannulated arterial preparation was placed into a cup-shaped glass bath and was perfused by a roller pump (Tokyo Rikakikai, Tokyo) with Krebs-Henseleit solution gassed with 95% O2 and 5% CO2. The solution contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, and 10 mM glucose. The flow rate was kept at approximately 2 ml/min. The perfusion pressure was continuously measured with an electric manometer (MPU-0.5A; Nihon Kohden, Tokyo) and recorded with a rectigraph (WT-685G, Nihon Kohden). After a stabilization period of 1 h, the preparation was removed from the bath solution and fixed in a horizontal position. The preparation was perfused at a constant flow rate during the experiment. The basal perfusion pressure was 40 – 80 mmHg.

For electrical stimulation of the periarterial sympathetic nerve terminals, two platinum electrodes were placed on the extraluminal side of the arterial wall. Electrical stimulation was delivered by an electric stimulator (SEN-7203, Nihon Kohden) using 30-s trains of pulses at 10 V amplitude, 1-ms pulse duration, and a frequency of 4 Hz. The organ bath was sealed with plastic films to maintain the preparation at 37°C. Ten-minute intervals between electrical stimulation periods were needed to obtain a reproducible response. Exogenous ATP and noradrenaline were administered into the rubber tubing close to the cannula in a volume of 0.01 – 0.03 ml, using microinjectors (Terumo, Tokyo).

Drugs

Drugs used were dl-noradrenaline hydrochloride (Sigma, St. Louis, MO, USA); disodium ATP (Sigma); angiotensin II acetate salt (synthetic, human sequence; Peptide Institute, Osaka); olmesartan (RNH-6270, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-((1-hydroxy-1-methylethyl)-2-propyl-1-(4-[2-(tetrazol-5-yl)-phenyl] phenyl)methylimidazol-5-carboxylate) (Sankyo Co., Ltd., Tokyo). Olmesartan was dissolved in 2.5% NaHCO3 aqueous solution and was further diluted to the concentrations used. Other drugs were dissolved in distilled water. Stock solutions were kept at –20°C until used.

Statistical analyses

Vasoconstrictor responses to electrical stimulation (PNS) are expressed as the maximum changes in perfusion pressure (mmHg) from their basal changes. The data are shown as the mean ± S.E.M. An analysis of variance with Bonferroni’s test was used for the statistical analysis of multiple comparisons of data. P values less than 0.05 were considered statistically significant.

Results

Effects of olmesartan on exogenous ATP- and noradrenaline-induced vasoconstrictions

A single bolus injection of ATP or noradrenaline induced a monophasic vasoconstrictor response in a concentration-related manner in isolated splenic arterial preparations. Perfusion of olmesartan at low concentrations (1 – 10 nM) did not induce any change in perfusion pressure (data not shown), but olmesartan at 100 nM slightly but insignificantly decreased the basal perfusion pressure from 38 ± 8 mmHg (control) to 32 ± 6 mmHg (after olmesartan) (n = 12, P>0.05). Exogenous ATP- and noradrenaline-induced vasoconstrictor responses were not influenced even by treatment with a relatively large dose of 100 nM olmesartan as shown in Fig. 1.

Effects of angiotensin II on vasoconstrictor responses to exogenously given ATP and noradrenaline

When a low concentration of 1 nM angiotensin II was applied intraluminally into the isolated and perfused canine splenic arterial preparation, it did not induce any vascular response. A higher concentration of angiotensin
II (10 nM) markedly increased the basal perfusion pressure over 100%, indicating its strong vasoconstrictor action, although a lower concentration of angiotensin II (1 nM) did not produce any potentiating effect on the vasoconstrictions of 0.01 – 1 μmol ATP and 0.03 – 3 nmol noradrenaline. Only at a high concentration of 10 nM did angiotensin II significantly potentiate either exogenous applied ATP- or noradrenaline-induced effects (Fig. 2).

**Effects of olmesartan on enhanced vasoconstrictor responses to ATP and noradrenaline by angiotensin II**

The potentiating effects of 10 nM angiotensin II on ATP- and noradrenaline-induced vasoconstrictions were significantly inhibited by the treatment with 10 nM olmesartan. After a high concentration of 100 nM olmesartan, ATP- and noradrenaline-induced responses returned to the almost control response levels, and the responses frequently were rather a little smaller than the control. Summarized data are shown in Fig. 2.

**Fig. 1.** No blocking effect of olmesartan on vasoconstrictions induced by exogenously administered ATP and noradrenaline in isolated and perfused canine splenic arteries. Data are presented as the mean ± S.E.M., n = 6.

**Fig. 2.** Potentiating effects of a relatively high concentration of angiotensin II on ATP (A)- and noradrenaline (B)-induced vasoconstrictions, and inhibitory effects of olmesartan on the potentiations. Data are presented as the mean ± S.E.M., n = 6. **P<0.01 vs angiotensin II-treated preparations.

**Effects of olmesartan on PNS-induced biphasic vasoconstrictor responses**

When periarterial nerve electrical stimulation was performed at 4 Hz, a double peaked vasoconstrictor response was readily induced as reported before (4, 5), showing a 1st and 2nd peaked vasoconstriction consisting of an initial transient constriction followed by a prolonged constrictor response in the isolated splenic artery.

In untreated preparations, PNS-induced biphasic responses (1st and 2nd peaked ones) were inhibited in parallel by olmesartan in a concentration-related manner. As shown in Fig. 3, olmesartan at 1 nM slightly inhibited the PNS-induced responses but not significantly. At 10 nM, olmesartan significantly inhibited both the 1st or 2nd peaked responses induced by PNS.

**Effects of olmesartan on PNS-induced constrictor responses potentiated by angiotensin II**

Figure 4a shows an exact tracing of the PNS-induced
biphasic responses. The response to PNS was markedly enhanced by treatment with 1 nM angiotensin II as shown in Fig. 4b. The potentiated responses were significantly inhibited by 10 nM olmesartan as shown in Fig. 4c. Moreover, an additional dose of 100 nM olmesartan suppressed the response to PNS more markedly than the control level as shown in Fig. 4d. Summarized data are shown in Fig. 5, indicating that a relatively low concentration of 1 nM angiotensin II markedly potentiated PNS-induced biphasic responses (**P<0.01) and olmesartan caused a strong antagonistic effect on the potentiated responses. The data showed that the PNS-induced vasoconstrictor responses after treatment with 1 nM angiotensin II are significantly inhibited by 100 nM olmesartan (##P<0.01).

Fig. 3. Effects of olmesartan on double peaked vasoconstrictions to PNS in canine splenic arteries. Data are presented as the mean ± S.E.M., n = 6. **P<0.01 vs control responses.

Fig. 4. Effects of olmesartan on angiotensin II-induced potentiating responses to PNS. a: Control biphasic response to PNS, b: Potentiated response by 1 nM angiotensin II, c: 10 nM olmesartan after response b, d: 100 nM olmesartan after response c. PNS, periarterial nerve electrical stimulation.

Fig. 5. Effects of olmesartan on angiotensin II-treated biphasic responses to PNS. Data are presented as the mean ± S.E.M., n = 8. **P<0.01 vs control response, #P<0.05, ##P<0.01 vs the angiotensin II-treated response.
Discussion

It has been recognized that the renin-angiotensin system (RAS) plays a key role in the regulation of blood pressure and fluid and electrolyte balance in mammals. Two angiotensin receptor subtypes (angiotensin II type 1 (AT$_1$) receptor and angiotensin II type 2 (AT$_2$) receptor) exist in a variety of tissues, and they have been cloned and characterized (9 – 12). The deleterious effects of angiotensin II (e.g., vasoconstriction and cardiac and vascular hypertrophy) are mediated by the AT$_1$ receptor, whereas the AT$_2$ receptor generally mediated opposing effects (13).

The introduction of angiotensin-converting enzyme (ACE) inhibitors has demonstrated the benefit of RAS blockade in the treatment of hypertension and congestive heart failure (14, 15). The treatment with ACE-inhibitors is, however, associated with adverse side effects such as cough, angioedema, and so on (16). Then, it had been considered that angiotensin receptor blocking agents might be useful to treat such cardiovascular diseases. Nonpeptide antagonists for the AT$_1$ receptor have been introduced for clinical use in the treatment of hypertension and have been successful (11). Losartan was followed by a large number of orally active AT$_1$ antagonists (7). In 1995, the novel nonpeptide angiotensin receptor antagonist CS-866 and its active form (olmesartan, RNH-6270) were reported (1). In the present study, we examined effects of olmesartan on vascular reactivity and angiotensin II-induced neuronal and vascular responses.

Previously we demonstrated that angiotensin II is involved in the modulation of purinergic and adrenergic vasoconstrictions to PNS in the canine splenic artery (17). It was reported that PNS induces a double peaked vasoconstriction consisting of an initial transient, predominantly P2X-purinoceptor-mediated response followed by a prolonged, mainly *a$_2$*-adrenoceptor-mediated one (4, 5). Angiotensin II in concentrations that did not produce any vascular effect markedly potentiated the double peaked responses to PNS but did not potentiate the responses induced by exogenously applied ATP and noradrenaline.

It has been reported that noradrenaline- and KCl-induced vasoconstrictions were enhanced by treatment with angiotensin II in the isolated canine mesenteric artery (20) in isolated rabbit femoral arterial rings (21) and in rabbit aortic rings (22). The potentiation might be due to an increase in intracellular sensitivity to Ca$^{2+}$, possibly mediated by protein kinase C (23). However, as shown in this study, the potentiating effects for exogenous ATP and noradrenaline were induced only by a high concentration of angiotensin II. Moreover, at a low concentration of angiotensin II, the PNS-induced responses were consistently and markedly potentiated. Thus, it is considered that the presynaptic action of angiotensin II might be physiological under in vivo conditions, but the postsynaptic action might occur in extremely high concentrations of angiotensin II.

In non-treated preparations as shown in Fig. 3, olmesartan consistently induced a decrease in the PNS-induced vasoconstriction in a concentration-related manner. Moreover, as shown in Fig. 5, even in angiotensin II-treated preparations, a relatively high concentration of olmesartan induced a decrease in the PNS-induced responses to much less than the control level, indicating that endogenous angiotensin II may readily stimulate AT$_1$ receptors in an isolated arterial preparation.

From these results, it is concluded that 1) olmesartan has selective AT$_1$ antagonistic properties, and 2) endogenous angiotensin II may readily exert its presynaptic stimulating action for ATP and noradrenaline releases from periartrial sympathetic nerve terminals.

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References


