

Inhibitory Effects of Olmesartan on Catecholamine Secretion from the Perfused Rat Adrenal Medulla

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The present study aimed to determine whether olmesartan, an angiotensin II (Ang II) type 1 (AT₁) receptor blocker, can influence the CA release from the isolated perfused model of the rat adrenal medulla. Olmesartan (5~50 μM) perfused into an adrenal vein for 90 min produced dose- and time-dependent inhibition of the CA secretory responses evoked by ACh (5.32 mM), high K⁺ (56 mM, a direct membrane-depolarizer), DMPP (100 μM) and McN-A-343 (100 μM). Olmesartan did not affect basal CA secretion. Also, in adrenal glands loaded with olmesartan (15 μM), the CA secretory responses evoked by Bay-K-8644 (10 μM, an activator of voltage-dependent L-type Ca²⁺ channels), cyclopiazonic acid (10 μM, an inhibitor of cytoplasmic Ca²⁺-ATPase), veratridine (100 μM, an activator of voltage-dependent Na⁺ channels), and Ang II (100 nM) were markedly inhibited. However, at high concentrations (150~300 μM), olmesartan rather enhanced the ACh-evoked CA secretion. Taken together, these results show that olmesartan at low concentrations inhibits the CA secretion evoked by cholinergic stimulation (both nicotinic and muscarinic receptors) as well as by direct membrane depolarization from the rat adrenal medulla, but at high concentrations it rather potentiates the ACh-evoked CA secretion. It seems that olmesartan has a dual action, acting as both agonist and antagonist at nicotinic receptors of the isolated perfused rat adrenal medulla, which might be dependent on the concentration. It is also thought that this inhibitory effect of olmesartan may be mediated by blocking the influx of both Na⁺ and Ca²⁺ into the rat adrenomedullary chromaffin cells as well as by inhibiting the Ca²⁺ release from the cytoplasmic calcium store, which is thought to be relevant to the AT₁ receptor blockade, in addition to its enhancement on the CA secretion.

Key Words: Olmesartan, Catecholamine secretion, Adrenal medulla, AT₁ receptor blockade

INTRODUCTION

Olmesartan medoxomil is an orally active, nonpeptide angiotensin receptor blockers (ARB) [1]. Olmesartan, the active metabolite of orally administered olmesartan medoxomil, binds with high selectivity to the angiotensin II (Ang II) type 1 (AT₁) receptor and bind to the type 2 (AT₂) receptor [2]. Binding of olmesartan to the AT₁ receptor in bovine tissue was >12 500-fold higher than binding to the AT₂ receptor [1,2]. Ang II is the primary effector peptide of the renin-angiotensin-aldosterone system (RAAS), which plays an important role in the regulation of blood pressure (BP) and fluid-electrolyte balance [3,4]. Activation of the AT₁ receptor by Ang II is responsible for all of the known cardiovascular (CV) effects of Ang II, and results in acute

vasoconstriction and increases in salt retention, fluid volume, aldosterone secretion and sympathetic activity [3,4], while activation of the AT₂ receptor is believed to result in the opposite effects. Olmesartan binds to the AT₁ receptor with a high degree of insurmountability and with greater affinity than most other ARBs [1,5,6].

Generally, the RAAS is an important mediator in the pathophysiology of hypertension, with excessive activity in the RAAS playing a key role in target end-organ damage, such as myocardial infarction, congestive heart failure, coronary artery disease and end-stage renal disease [7]. Thus, this system is a prime target for drugs used in the treatment of hypertension, with two classes of antihypertensive agents targeting the RAAS. The first class, ACE inhibitors (e.g. enalapril, captopril), block the conversion of Ang I to Ang II via inhibition of angiotensin converting enzyme; however, this pathway is only one of several pathways that are involved in the synthesis of Ang II and thus the synthesis of Ang II is only partially blocked [8]. The second

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ABBREVIATIONS: ACh, acetylcholine; Bay-K-8644, methyl-1, 4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methyl-phenyl)-pyridine-5-carboxylate; CA, catecholamines; DMPP, 1,1-dimethyl-4-phenyl piperazine iodide; McN-A-343, 3-(m-chloro-phenyl)-carbamoyl-oxy-2-butynyl-trimethyl ammonium chloride; NO, nitric oxide.

class of agents, the Ang II receptor antagonists (ARBs), which includes olmesartan medoxomil, block the RAAS more completely through antagonism of Ang II binding to the Ang II type 1 (AT₁) receptor, thereby inhibiting the vasoconstrictor and aldosterone-secreting effects of Ang II [8].

The AT₁ antagonist olmesartan blocked both inhibition and facilitation of secretion by Ang II in cultured bovine chromaffin cells [9], and chronic blockade (olmesartan) of the RAAS in rats may decrease the excess sympathetic responses to stress in cardiovascular diseases as well as prevent the likely development of Type II diabetes mellitus [10]. In spontaneously hypertensive rats (SHRs), oral administration of AT₁ antagonist (candesartan) can effectively block central actions of Ang II, regulating blood pressure and reaction to stress, and selectively and differentially modulating sympathoadrenal responses [11]. Critchley and his colleagues [12] have found that AT₁ receptor antagonist candesartan, and the ACE inhibitor ramipril, increased basal CA release from the anaesthetized dog's adrenal gland along with decreases in blood pressure.

However, it has been shown that AT₂ stimulation induces CA secretion in cultured porcine chromaffin cells [13]. This suggests that AT₂ receptors play a role in mediating CA secretion from the adrenal medulla of anesthetized dogs in response to Ang II receptor agonist administration *in vivo*. Furthermore, both PD 123319 and CGP 42112 inhibited the increase in adrenal CA secretion induced by local administration of Ang II [14]. Worck and his colleagues [15] have speculated that Ang II through binding to both receptor subtypes (both AT₁ and AT₂) facilitates the sympathoadrenal reflex response by actions at several anatomical levels of the neural pathways involved in the sympathoadrenal reflex response elicited during insulin-induced hypoglycemia in conscious chronically instrumented rats.

Thus, there seems to be some controversy about the effect of AT₁ receptor blockade on the CA secretion in the adrenal gland. There is no direct evidence of olmesartan's effects on the adrenal CA secretion so far. The aim of this study therefore was to determine whether olmesartan, a selective antagonist of AT₁ receptor, could influence the CA release in the isolated perfused model of the rat adrenal medulla.

METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 200 to 300 grams, were anesthetized with thiopental sodium (50 mg/kg) intraperitoneally. The adrenal gland was isolated by the methods described previously [16]. The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed with the placement of three-hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered with saline-soaked gauge pads and urine in the bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite the entrance of the adrenal vein. Perfusion

of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in the adrenal cortex. Then the adrenal gland, along with the ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37±1°C.

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic pump (ISCO[®] pump, WIZ Co. U.S.A.) at a rate of 0.32 ml/min. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.18; NaHCO₃, 25; KH₂PO₄, 1.2; glucose, 11.7. The solution was constantly bubbled with 95% O₂+5% CO₂ and the final pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 µg/ml) and ascorbic acid (100 µg/ml) to prevent oxidation of catecholamines.

Drug administration

The perfusions of DMPP (100 µM) and Ang II for 1 or 2 minutes and/or a single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into the perfusion stream via a three-way stopcock, respectively. McN-A-343 (100 µM), veratridine (100 µM), Ang II (100 nM), Bay-K-8644 (10 µM) and cyclopiazonic acid (10 µM) were also perfused for 4 min, respectively.

In the preliminary experiments, it was found that upon administration of the above drugs, the secretory responses to ACh, KCl, McN-A-343, veratridine, Ang II, Bay-K-8644 and cyclopiazonic acid returned to preinjection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

As a rule, prior to stimulation with the various secretagogues, the perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's perfusate was collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from that secreted from the stimulated sample to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effect of olmesartan on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing olmesartan for 60 min, and then the perfusate was collected for a certain period (background sample). Then the medium was changed to the one containing the stimulating agent or along with olmesartan, and the perfusates were collected for the same period as that for the background sample. The adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

The CA content of the perfusate was measured directly by the fluorometric method of Anton and Sayre [17] without the intermediate purification alumina for the reasons described earlier [18] using a fluorospectrophotometer (Kontron

Co., Milano, Italy).

A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of the stimulated glands by secretagogues used in the present work was high enough to obtain readings several folds greater than the reading of the control samples (unstimulated). The sample blanks were also lowest for perfusates of the stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical difference between the control and pre-treated groups was determined by the Student's *t* and ANOVA tests. A *p* value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (SEM). The statistical analysis of the experimental results was made using a computer program described by Tallarida and Murray [18].

Drugs and their sources

The following drugs were used: olmesartan medoxomil (a gift from Daiichi Sankyo Co., Ltd, Japan), cyclopiazonic acid, acetylcholine chloride, 1,1-dimethyl-4-phenyl piperazine iodide (DMPP), norepinephrine bitartrate, angiotensin II methyl-1, 4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methyl-phenyl)-pyridine-5-carboxylate (Bay-K-8644), veratridine hydrochloride, (Sigma Chemical Co., U.S.A.), and (3-(*m*-chloro-phenyl-carbamoyl-oxy)-2-butynyltrimethyl ammonium chloride [McN-A-343]) (RBI, U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which was dissolved in 99.5% ethanol and diluted appropriately with Krebs-bicarbonate solution (final concentration of alcohol was less than 0.1%). Concentrations of all drugs are expressed in terms of their molar base.

RESULTS

Effects of olmesartan on CA secretion evoked by ACh, high K⁺, DMPP and McN-A-343 from the perfused rat adrenal glands

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 20 ± 2 ng for 2 min ($n=12$). Since previous studies have indicated that the ARBs, which includes olmesartan medoxomil, block the RAAS more completely through antagonism of Ang II binding to the Ang II type 1 (AT₁) receptor, thereby inhibiting the vasoconstrictor and aldosterone-secreting effects of Ang II [8], and that olmesartan dose-dependently antagonizes Ang II-induced contractions in isolated guinea pig aortic tissue and inhibits the pressor response to intravenously administered Ang II in normotensive rats [2], it was attempted initially to examine the effects of olmesartan itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, olmesartan (5~50 μ M) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of olme-

sartan on cholinergic receptor stimulation— as well as membrane depolarization—mediated CA secretion. Secretagogues were given at 15 min-intervals. Olmesartan was present for 90 minutes after the establishment of the control release.

When ACh (5.32 mM) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was $1,277 \pm 32$ ng for 4 min. However, in the presence of olmesartan in the range of 5~50 μ M for 90 min, ACh-stimulated CA secretion was inhibited in both a concentration- and time-dependent fashion. As shown in Fig. 1 (upper), in the presence of olmesartan, CA releasing responses were inhibited to 61% of the corresponding control release. Also, the depolarizing agent, high potassium, markedly stimulated the CA secretion (791 ± 31 ng for 0~4 min). However, following the pretreatment with olmesartan (5~50 μ M), high K⁺ (56 mM)—stimulated CA secretion was significantly inhibited to 54% of the control at last period (75~94 min)

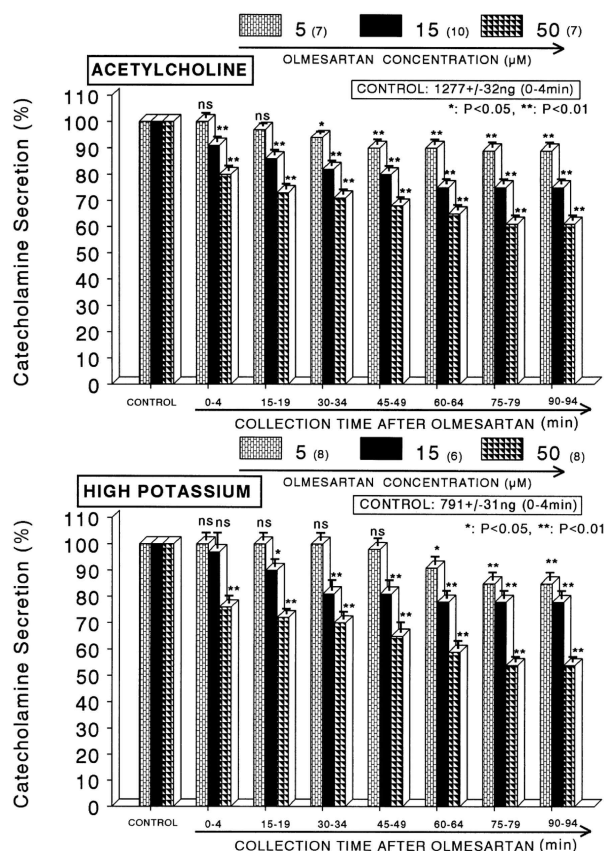


Fig. 1. Dose-dependent effects of olmesartan on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh, upper) and high potassium (lower) from the perfused rat adrenal medullas. The CA secretion by a single injection of ACh (5.32 mM) and K⁺ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 5, 15 and 50 μ M of olmesartan for 90 min as indicated at an arrow mark, respectively. Numbers in the parenthesis indicate number of rat adrenal glands. Vertical bars on the columns represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland (% of control). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-pretreated group of olmesartan. ACh- and high K⁺-induced perfusates were collected for 4 minutes, respectively. **p* < 0.05, ***p* < 0.01. ns: Statistically not significant.

as shown in Fig. 1 (lower). DMPP (100 μ M), which is a selective nicotinic (N_N) receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion ($1,259 \pm 28$ ng for 0~8 min). However, as shown in Fig. 2 (upper), DMPP-evoked CA secretion after pretreatment with olmesartan was greatly reduced to 60% of the control release (100%). McN-A-343 (100 μ M), which is a selective muscarinic M_1 -receptor agonist [19], perfused into an adrenal gland for 4 min also caused an increased CA secretion (614 ± 23 ng for 0~4 min). However, in the presence of olmesartan, McN-A-343-evoked CA secretion was markedly depressed to 56% of the corresponding control secretion (100%) as depicted in Fig. 2 (lower).

Effect of olmesartan on CA secretion evoked by Bay-K-8644, cyclopiazonic acid, veratridine and Ang II from the perfused rat adrenal glands

Since Bay-K-8644 is known to be a calcium channel acti-

vator, which enhances basal Ca^{2+} uptake [20] and CA release [21], it was of interest to determine the effect of olmesartan on Bay-K-8644-evoked CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10 μ M)-evoked CA secretion in the presence of olmesartan (15 μ M) was greatly blocked to 75% of the control at 75~94 min period as compared to the corresponding control release (614 ± 17 ng for 0~4 min) from 10 adrenal glands as shown in Fig. 3 (upper).

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+} -ATPase in the skeletal muscle sarcoplasmic reticulum [22,23]. The inhibitory action of olmesartan on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 3 (lower). In the presence of olmesartan (15 μ M) from 8 adrenal glands, cyclopiazonic acid (10 μ M)-evoked CA secretion was also inhibited to 68% of the control response (544 ± 21 ng for 0~4 min).

The voltage-dependent Na^+ channels consist of the principal α -subunit, which is associated with noncovalently attached β_1 -subunits, and a disulfide-linked β_2 -subunit [24]. It has also been known that veratridine-induced Na^+ influx

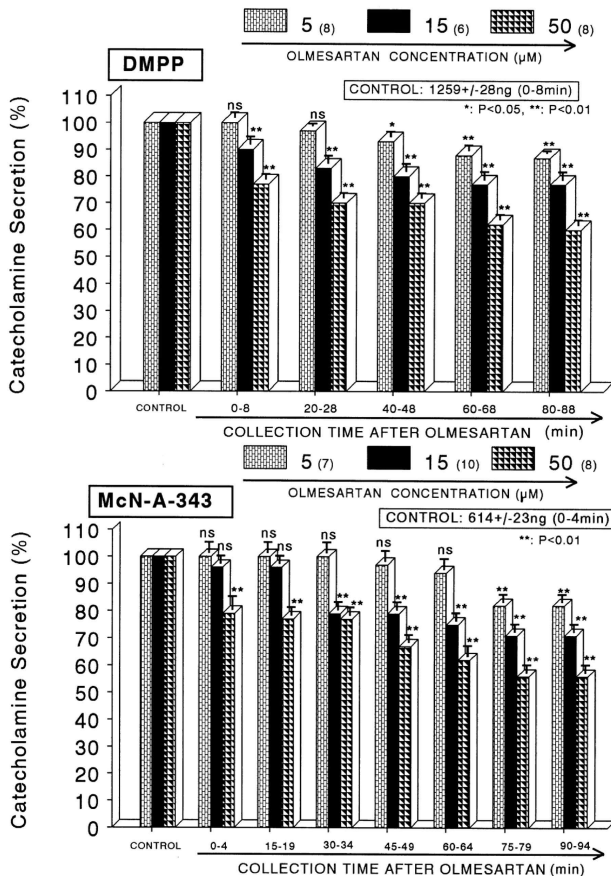


Fig. 2. Dose-dependent effects of losartan on the CA secretory responses evoked by DMPP (upper) and McN-A-343 (lower) from the perfused rat adrenal medullas. The CA secretion by perfusion of DMPP (100 μ M) and McN-A-343 (100 μ M) for 2 min and 4 min was induced at 20 and 15 min intervals after preloading with 5, 15 and 50 μ M of olmesartan for 90 min, respectively. Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-pretreated group of olmesartan. DMPP- and McN-A-343-induced perfusates were collected for 8 and 4 minutes, respectively. Other legends are the same as in Fig. 1. * $p < 0.05$, ** $p < 0.01$. ns: Statistically not significant.

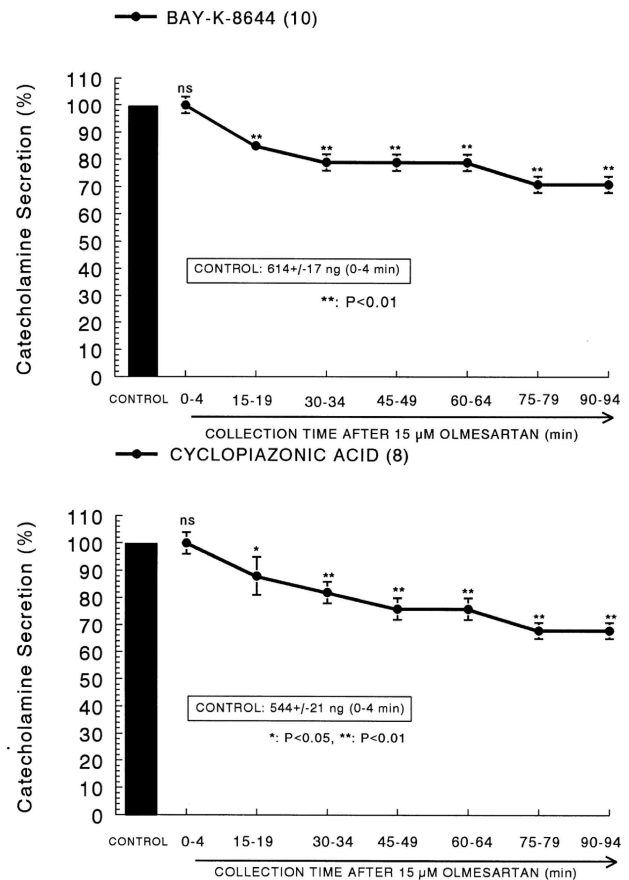


Fig. 3. Time-course effects of olmesartan on the CA release evoked by Bay-K-8644 (upper) and cyclopiazonic acid (lower) from the perfused rat adrenal medullas. Bay-K-8644 (10 μ M) and cyclopiazonic acid (10 μ M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with olmesartan (15 μ M) for 90 min, respectively. Other legends are the same as in Fig. 1. * $p < 0.05$, ** $p < 0.01$. ns: Statistically not significant.

mediated through Na^+ channels increased Ca^{2+} influx via activation of voltage-dependent Ca^{2+} channels and produced the exocytotic secretion of CA in cultured bovine adrenal medullary cells [25]. To characterize the pharmacological action of olmesartan on voltage-dependent Na^+ channels, the effect of olmesartan on the CA secretion induced by veratridine was examined here. As shown in Fig. 4 (upper), veratridine greatly produced CA secretion ($1,331 \pm 21$ ng for 0~4 min). However, in the presence of olmesartan ($15 \mu\text{M}$), veratridine ($100 \mu\text{M}$)–evoked CA secretion was greatly inhibited to 63% of the corresponding control release.

Since Hano and his colleagues [26] have suggested that Ang II increase epinephrine release from the adrenal medulla via the AT_1 receptors, it was likely interesting to examine the effect of Ang II on the CA release. Ang II (100 nM) significantly evoked the CA secretory response (585 ± 26 ng for 0~4 min) whereas, in the presence of olmesartan ($15 \mu\text{M}$), Ang II (100 nM)–evoked CA secretion was greatly inhibited to 43% of the corresponding control release (Fig. 4–lower).

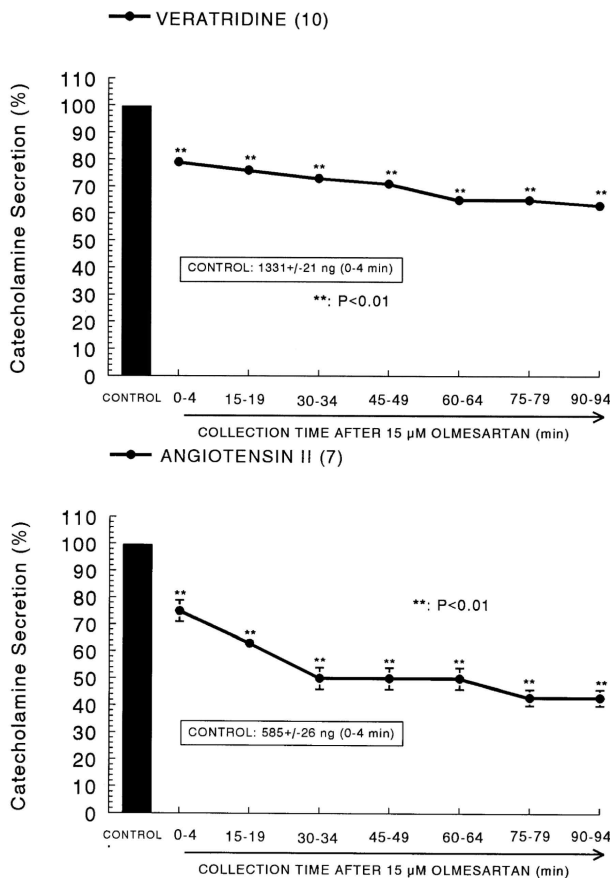


Fig. 4. Time–course effects of olmesartan on the CA release evoked by veratridine (upper) and angiotensin II (lower) from the perfused rat adrenal medullas. Veratridine ($100 \mu\text{M}$) and angiotensin II (100 nM) was perfused into an adrenal vein for 4 min and 1 min at 15 min intervals after preloading with olmesartan ($15 \mu\text{M}$) for 90 min, respectively. Other legends are the same as in Fig. 1. ** $p < 0.01$.

High dose effects of olmesartan on CA release evoked by ACh from the perfused rat adrenal glands

As shown in Fig. 1~4, it has also been shown that olmesartan inhibits the CA secretory response evoked by several secretagogues in the perfused rat adrenal glands. Therefore, in order to study the high dose effects of olmesartan on the CA secretion, in the presence of high doses (150 and $300 \mu\text{M}$) of olmesartan, the CA secretory responses evoked by ACh–stimulation were examined. In the presence of olmesartan ($150 \mu\text{M}$) for 90 min, ACh–evoked CA release was not affected at initial periods (0~49 min), but since then significantly enhanced to 108% of the corresponding control release as illustrated in Fig. 5. Moreover, after treatment with higher concentration ($300 \mu\text{M}$) for 90 min, ACh–evoked CA release was greatly enhanced to 117% of the corresponding control release during all periods (Fig. 5).

DISCUSSION

The main findings of the present study have demonstrated that olmesartan can inhibit the CA secretion evoked by cholinergic (both nicotinic and muscarinic receptors) stimulation and direct membrane–depolarization from the rat adrenal medulla. This inhibitory effect of olmesartan seems to be mediated by blocking the influx of Na^+ and Ca^{2+} ions through their channels as well as by inhibiting the release of Ca^{2+} from cytoplasmic store through the blockade of AT_1 receptors located on the presynatic membrane of the rat adrenomedullary chromaffin cells, which are relevant to adrenal nicotinic receptor blockade.

In the adrenal medulla, Ang II releases the CA by a direct action [27], mediated either by circulating Ang II or by the intrinsic RAAS [27–29]. Both AT_1 and AT_2 Ang II receptors are expressed in the adrenal medulla. In the rat, AT_2 re–

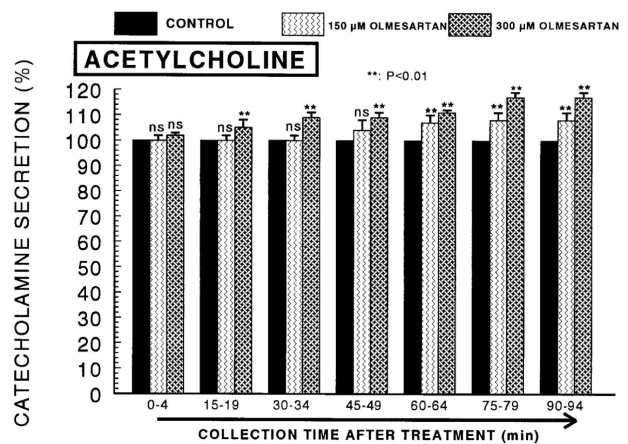


Fig. 5. High dose–effects of olmesartan on the ACh–evoked CA secretory responses from the perfused rat adrenal medullas. The CA secretion by a single injection of ACh (5.32 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 150 and $300 \mu\text{M}$ of olmesartan for 90 min as indicated at an arrow mark. ACh–induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 1. ** $p < 0.01$. ns: Statistically not significant.

ceptors predominate, AT₁ receptors representing only 5~10% of the total number of Ang II receptors [30]. It appears that AT₁ receptor stimulation is most important as a regulatory factor for adrenomedullary CA synthesis and release. First, blocking AT₁ is sufficient to inhibit *in vivo* adrenal CA release by Ang II [31]. Second, pretreatment with an insurmountable AT₁ antagonist almost completely abolished the hormonal and sympathoadrenal response to the stress of isolation in unfamiliar metabolic cages [32].

In support of the present results, previously, Armando and his colleagues [32] found that pre-treatment with candesartan, an ARB, eliminated the increase in adrenal nor-epinephrine (NE) and epinephrine (E) concentrations induced by isolation stress. On the other hand, it has been shown that acute and chronic stress stimulates the RAAS to increase the levels of Ang II, both in the plasma and brain [33]. Uresin and his colleagues [10] have also speculated that chronic blockade (losartan) of RAAS in rats may decrease the excess sympathetic responses to stress in cardiovascular diseases and prevent the likely development of Type II diabetes mellitus. Olmesartan, by blocking the interaction of Ang II with its AT₁ receptor, dose-dependently antagonized Ang II-induced contractions in isolated guinea pig aortic tissue and inhibited the pressor response to intravenously administered Ang II in normotensive rats [2]. Olmesartan medoxomil is an orally active, nonpeptide ARB [1]. Olmesartan, the active metabolite of orally administered olmesartan medoxomil, binds with high selectivity to the AT₁ receptor and bind to the type 2 (AT₂) receptor [2]. Binding of olmesartan to the AT₁ receptor in bovine tissue was >12 500-fold higher than binding to the AT₂ receptor [1,2]. Olmesartan binds to the AT₁ receptor with a high degree of insurmountability and with greater affinity than most other ARBs [1,5,6].

Furthermore, the AT₁ antagonist, losartan blocked both inhibition and facilitation of secretion by AngII in cultured bovine chromaffin cells [9]. The results of this study showed that activation of multiple types of G-proteins and transduction pathways by single neuromodulator acting through one receptor type can produce concentration-dependent, bi-directional regulation of exocytosis [9]. Based on previous findings, the present results that olmesartan dose- and time-dependently reduced the CA secretory responses evoked by ACh, high potassium, DMPP and McN-A-343 from the perfused rat adrenal medulla might be due to the blockade of AT₁ receptors located presynaptically on rat adrenomedullary chromaffin cells. Moreover, it has been shown that, in spontaneously hypertensive rats (SHRs), oral administration of AT₁ antagonist (candesartan) can effectively block central actions of Ang II, regulating blood pressure and reaction to stress, and selectively and differentially modulating sympathoadrenal response and the hypothalamic-pituitary-adrenal stimulation produced by brain Ang II [11]. In the present study, as shown in Fig. 4 (lower), olmesartan also greatly inhibited Ang II-evoked CA release from the rat adrenal medulla. This finding indicates that olmesartan can inhibit the CA release evoked by cholinergic stimulation as well as by membrane depolarization through the AT₁ receptor blockade.

The nicotinic receptor is a neurotransmitter-gated cation-conducting ion channel that is opened by binding of agonists such as ACh and DMPP [34]. The opening of this channel triggers Ca²⁺ uptake and secretion of CA from chromaffin cells [25]. To determine if the inhibition of DMPP-stimulated secretion by AT₁ antagonist was due to

an effect on the activity of the nicotinic receptor, the effect of olmesartan, an AT₁-selective agonist, on DMPP-stimulated CA secretion was examined. As shown in Fig. 2, treatment with olmesartan greatly inhibited DMPP-evoked CA secretion, reducing by 71% of the control release. The present data are similar to the result that chronic immobilization stress increased plasma glucose, NE, E and corticosterone levels in the rats, and that the ARB losartan significantly prevented these increments induced by chronic stress when given before the stress regimen [10]. Therefore, it is likely plausible that olmesartan can alter the activity of both nicotinic receptors and voltage-sensitive Na⁺ channels.

In the present study, olmesartan, an AT₁-selective antagonist inhibited the CA secretory responses by high potassium, a direct membrane depolarizer, as well as by Bay-K-8644, an activator of voltage-dependent L-type Ca²⁺ channels, which facilitates the influx of Ca²⁺ into the cells. The observation that AT₁-selective antagonist inhibited the CA secretion evoked by Bay-K-8644 was surprising, as Takekoshi et al. [13] have reported that removal of external Ca²⁺ significantly suppressed either AngII plus CV-11974 (AT₁ antagonist, 100 nM; which simulates specific AT₂ stimulation) or CGP 42112 (AT₂ agonist)-induced CA secretion in cultured porcine adrenomedullary chromaffin cells. It is unclear how the blockade of AT₁ receptors results in the inhibition of secretion seen in these cells. In the present work, the simplest interpretation is that the decrease in Ca²⁺ uptake by olmesartan is responsible for the observed inhibition of the CA secretion. However, such an interpretation is complicated by the complexity of the relationship between the CA secretion and intracellular free Ca²⁺ levels. Both the intracellular location of the Ca²⁺ level increase [35,36] and the magnitude of the Ca²⁺ level increase [37] can affect the relationship between intracellular free Ca²⁺ levels and secretion. Holz et al. [37] have reported that when Ca²⁺ uptake is large, changes in Ca²⁺ uptake resulted in less than proportional changes in CA secretion. Consequently, although the decrease in Ca²⁺ uptake (influx) into the adrenal chromaffin cells may explain the decrease by olmesartan in CA secretion, it is still unclear whether this is only or even most important factor contributing to the inhibition of CA secretion by olmesartan. However, in view of the results so far obtained from the present study, it is felt that the voltage-sensitive Ca²⁺ channels located on chromaffin cell membrane of the rat adrenal medulla could be the target site for olmesartan-mediated inhibition of CA secretion.

In the present study, olmesartan also inhibited the CA secretory responses evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca²⁺-ATPase in skeletal muscle sarcoplasmic reticulum [22,23]. Therefore, it is felt that the inhibitory effect of olmesartan on the CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca²⁺ in the chromaffin cells. This indicates that olmesartan-induced blockade of AT₁ receptors causes an inhibitory effect on the release of Ca²⁺ from the intracellular pools induced by stimulation of muscarinic ACh receptors, which is weakly responsible for the secretion of CA. In the present work, olmesartan time- and concentration-dependently produced the inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M₁-agonist. This fact suggests new other concept that olmesartan can modulate the CA secretory process induced by

activation of muscarinic M_1 -receptors as well as neuronal nicotinic receptors in the rat adrenal medulla. In support of this finding, it has been shown that cyclopiazonic acid easily penetrates into the cytoplasm through the plasma membrane and reduces Ca^{2+} -ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in increase in the subsequent Ca^{2+} release from those storage sites and thereby increase of Ca^{2+} -dependent K^+ -current [38]. Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors is also proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1,4,5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools [35,39]. However, in the present study, it is uncertain whether the inhibitory effect of the olmesartan on Ca^{2+} movement from intracellular pools is due to their direct effect on the PI response or the indirect effect as a result of AT_1 receptor blockade by olmesartan. Based on these previous results, this finding of the present work suggests that olmesartan-induced inhibition can regulate the CA secretion evoked by muscarinic M_1 -receptor stimulation in the rat adrenal medullary chromaffin cells. Furthermore, Ang II is a secretagogue for CA release that is believed to be mediated through IP_3 production by AT_1 [31,40]. Indeed, Wong and his colleagues [31] demonstrated that AngII-induced CA release is mediated by AT_1 in the rat adrenal medulla. AT_1 -mediated phospholipase C activation and subsequent IP_3 formation may increase cytosolic Ca^{2+} levels by releasing Ca^{2+} from intracellular storage, with subsequent activation of CA release [30]. Indeed, it has been shown that addition of IP_3 to permeabilized bovine chromaffin cells releases intracellular Ca^{2+} [41]. Furthermore, addition of Ca^{2+} to permeabilized bovine chromaffin cells was reported to cause CA secretion [42].

On the other hand, in the present work, high concentrations of olmesartan (150 and 300 μM) significantly enhanced ACh-evoked CA secretory responses. As this result alone, there seems to be difficult for interpretation of the enhancement of ACh-evoked CA secretion by high dose of olmesartan. In support of this idea, the research results of Vijayapandi and Nagappa [43] showed biphasic effects of losartan potassium on immobility in mice: reduced immobility at lower dose (0.1 and 5 mg/kg, i.p.) and enhanced immobility in higher dose (100 mg/kg, i.p.). These biphasic effects were further confirmed by interaction of losartan potassium with reserpine and antidepressant drugs, nortriptyline and fluoxetine [43]. Nahmod and his colleagues [44] found Ang II to cause 5-HT release and accelerate its synthesis in biphasic manner, stimulating at high doses and inhibiting at lower doses. Vijayapandi and Nagappa [43] have obtained that the biphasic effect of losartan potassium on immobility in mice might be due to inhibitory effect on AT_1 receptor at lower dose and pronounced effect on AT_2 receptor at higher dose (large concentrations of losartan potassium can displace Ang II from its AT_1 receptor to AT_2 receptor). In chronic studies with losartan potassium even at lower dose (3 mg/kg, P.O.) potentiated immobility in mice, which might be due to continuous blockade of AT_1 receptor resulting in unopposed AT_2 receptor stimulation [43]. It has also been previously reported that the treatment of Ang II for 4 h has a biphasic effect on Na^+ transport in the primary cultured rabbit renal proximal tubule cells (PTCs); a pico molar range of Ang II stimulates Na^+ transport, whereas a micro molar range of Ang II inhibits it [45]. Based on these previous results, in the present

study, it seems that biphasic effects of olmesartan on the CA secretion in the perfused rat adrenal medulla are due to inhibitory effect on AT_1 receptor at lower dose (5~50 μM) and pronounced effect on AT_2 receptor at higher dose (150 and 300 μM), indicating that large concentrations of olmesartan can displace Ang II from its AT_1 receptor to AT_2 receptor. However, the detailed relationship between AT_1 and AT_2 receptors in adrenomedullary CA secretion should be confirmed in the future study.

Collectively, the present work has demonstrated that olmesartan at low concentrations inhibits the CA secretion evoked by cholinergic (both nicotinic and muscarinic receptors) stimulation as well as by direct membrane-depolarization from the rat adrenal medulla, but at high concentration it rather enhances ACh-evoked CA secretion. It seems that olmesartan has dual action acting as both agonist and antagonist at nicotinic receptors of the rat adrenal medulla, which might be dependent on the concentration. It is also thought that this olmesartan-induced inhibition may be mediated by blocking the influx of both Na^+ and Ca^{2+} ions through their channels into the rat adrenomedullary chromaffin cells as well as by inhibiting the Ca^{2+} release from its cytoplasmic calcium store, which is thought to be relevant to AT_1 receptor blockade, in addition to its unknown enhancement effect on the CA release.

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