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Influence of ACE Inhibitor on Nicotinic Stimulation-Evoked Catecholamine Secretion in the Perfused Rat Adrenal Medulla

조선대학교 대학원

의학과

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안지오텐신-전환효소 억제제가 흰쥐 부신수질에서 니코틴 흥분작용에 의한 카테콜아민 분비에 미치는 영향

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서유석

서 유 석의 박사학위논문을 인준함

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서 유 석

(지도교수: 임 동 윤)

조선대학교 대학원 의학과

Critchley 등 (1988) 과 MacLean 및 Ungar (1986)가 부신수질에서 안지오텐신 전환효소(ACE) 억제제 (enalapril 과 captopril)의 생체내 효과를 처음 연구하였으며, 이들은 개의 부신에서 내장신경 자극에 의한 카테콜아민(CA) 유리를 억제한다고 하였다. 또한, Armando 등 (2004)은 부신수질의 AT_1 및 AT_2 수용체 모두가 CA 합성 및 티로신 수산화효소의 전사조절을 유지하고 촉진시킨다고 하였다. 한편 사람의 부신크롬친화세포에서 candesartan 은 안지오텐신 II (AngII)에 의한 세포내 칼슘 증가를 차단하는 반면, AT_2 수용체 작동제인 T_2-(Ang II 4-8)$_2$ 는 아무런 영향을 미치지 못한다고 하였다 (Cavadas 등, 2003). 이와 같이 부신에서 ACE 억제제와 AT_1 차단제의 CA 분비작용에 관해서 일부 논란이 있는 것 같다. 따라서 본 연구의 목적은 흰쥐 부신의 적출관류모델에서 ACE 억제제가 카테콜아민 유리에 영향을 미치는지를 검색하고, 나아가 ACE 억제제인 enalapril 과 선택성 AT_1 차단제인 losartan 을 병용 처치하여

CA 분비작용을 비교코자 본 연구를 시행하여 다음과 같은 결과를 얻었다. Enalapril (50-500 μM)을 부신정맥 내로 90 분간 관류 시 용량 및 시간 의존적으로 ACh ($5.32x10^{-3}$ M), 고칼륨 ($5.6x10^{-2}$ M), DMPP (10^{-4} M) 및 McN-A-343 (10^{-4} M)에 의한 CA 분비반응을 유의하게 억제하였다. 또한, 90 분 동안 150 μM enalapril 존재 하에서, L 형 칼슘통로 활성화제인 Bay-K-8644 (10^{-5} M), 세포질의 내형질세망막에서 Ca^{2+}-ATPase 억제제인 cyclopiazonic acid (10^{-5} M), 선택성 나트륨통로 활성화제인 veratridine (10^{-4} M) 및 angiotensin II (Ang II, 10^{-7} M)에 의한 CA 분비반응이 뚜렷이 억제되었다. 또한, 또 다른 ACE 억제제인 captopril (150 μM)은 상기 분비자극제에 의한 CA 분비반응에 대해 유사한 시간의존성 억제효과를 나타내었다. Enalapril 및 captopril 자체는 기초 CA 분비량에 영향을 미치지 않았다. Enalapril (150 μM) 와 losartan (15 μM) 동시 존재 하에서 ACh, 고칼륨, DMPP 및 McN-A-343, 그리고 Bay-K-8644, cyclopiazonic acid, veratridine 및 AngII 의 CA 분비에 대하여 enalapril (150 μM) 이나 losartan (15 μM) 단독처치 시와 비교하여 억제작용이 유의하게 증강되었다. 이와 같은 연구결과를 종합하여 보면, 흰쥐 적출 관류 부신수질에서 enalapril 과 losartan 은 다같이 AngII 뿐만 아니라 콜린성(니코틴 및 무스카린 수용체) 흥분작용 및 직접막탈분극에 의한 CA 분비작용을 유의하게 억제하였으며, enalapril 과 captopril 은 다같이 부신 크롬친화세포의 니코틴수용체에서 길항작용을 갖는 것으로 생각된다. 이러한 enalapril 과 captopril 의 CA 분비 억제작용은 전압의존성 Na^{+} 및 Ca^{2+} 이온통로를 통한 부신수질의 크롬친화세포내로 나트륨 및

칼슘이온의 유입을 차단하고 세포질내 칼슘저장고로부터 칼슘유리의 억제작용을 통해 매개되는 것으로 사료되며, 이는 국소적인 ACE 억제작용과 관련이 있는 것으로 생각된다. ACE 억제제와 AT_1 차단제의 병용시 CA 분비 억제작용은 증강되는 것으로 사료된다. 본 연구의 결과로 보아, 흰쥐 관류 부신수질의 CA 분비에 ACE 가 관여하며, 고혈압, 심장부전 및 콩팥부전과 같은 삼혈관계 질환을 조절하는 데 ACE 억제제와 AT_1 차단제의 병용이 ACE 억제제와 AT_1 차단제의 단독투여에 비해서 유용한 이점이 있는 것으로 생각된다.

I. INTRODUCTION

The angiotensin-converting-enzyme (ACE) has also been localized in the glomerulosa zone of the cortex and in the medulla of adrenal gland (Chai et al., 1986; Israel et al., 1986; Strittmatter et al., 1986; Oda et al., 1991). It is generally accepted that the adrenal gland contains all of the components required for the normal function of local renin-angiotensin system (RAS) (Nakamura et al., 1985; Chai et al., 1986). On the other hand, the increased secretion of adrenal catecholamines (CA) induced by AngI was attributed to a direct action of angiotensin-I (AngI) on adrenal chromaffin cells in anesthetized cats (Peach, 1971; Peach et al., 1971). However, it has been shown that the adrenal CA secretion in response to hemorrhage was attenuated by captopril (an ACE inhibitor) in anesthetized cats (Feuerstein and Cohen, 1979) and dogs (Kimura et al., 1992). McGrath and Arnolda (1986) have suggested that ACE inhibition with enalapril reduces sympathetic activity in patients with heart failure. ACE inhibition is accompanied by a decrease in plasma CA concentrations in patients with essential hypertension (Giannattasio, et al., 1992), suggesting that the drugs reduce sympathetic activity.

The ACE inhibition with enalapril has been shown to produce a sympatho-inhibitory effect in human hypertensives (Minatoguchi et al., 1992). One possible explanation is that enalapril reduces angiotensin-II (AngII) formation thus decreasing the activation of release-enhancing AngII receptors on postganglionic sympathetic nerve endings (Minatoguchi et al., 1992). Experimental studies have shown that ACE inhibitors have positive effects on the

circulation. Enalapril maleate, which is an ACE inhibitor, is used as an antihypertensive drug and blocks AngI conversion to AngII by its action on ACE (Murat and Ecemis, 1995; Katzung, 1995; Birincioglu et al., 1997). AngII elevates the release of norepinephrine from terminal adrenergic neurons. (Birincioglu et al., 1997; Forster and Le Tran, 1996) Moreover, AngII elevates the release of epinephrine and norepinephrine from adrenal medulla and stimulates the autonomic ganglions in the peripheral autonomic nerve system (Murat and Ecemis, 1995; Forster and Le Tran, 1996; Le Tran and Forster, 1996). It also has been shown that the local ACE is functionally involved in regional AngII formation in the adrenal gland in anesthetized dogs. This study suggests that AngII thus generated may play a role in the local regulation of adrenal CA secretion (Yamaguchi et al., 1999).

The *in vivo* effects of the ACE inhibitors (enalapril and captopril) on the adrenal medulla were first investigated by Critchley et al. (1988) and MacLean and Ungar (1986) who showed that they inhibited CA release during splanchnic nerve stimulation of the dog adrenal gland. Martineau and his colleagues (1995; 1999) have investigated the effects of a number of AngII type 1 (AT_1) antagonists on AngII-induced CA release from the anesthetized dog's adrenal gland. Recently, enalapril maleate has been shown to block the effect of cold stress on the regulation of tyrosine hydroxylase (TH) activity in adrenal medulla, heart and hypothalamus in rat (Talas and Yurekli, 2006). While these studies suggest the functional involvement of circulating AngII in controlling release of adrenal CA, whether the local conversion of AngI to AngII takes place within the isolated perfused model of adrenal gland remains unclear. At present, functional *in vitro* studies on the potential involvement of local ACE in the adrenal gland are limited

in number.

The present study therefore was designed to examine whether ACE inhibitor can affect the CA release from the perfused model of the isolated rat adrenal gland, and also to compare the *in vitro* combined effects of an AT_1 receptor antagonist, losartan, and an ACE inhibitor, enalapril maleate, on adrenal CA release, because these two classes of drugs have not been compared in respect to their effects on adrenal CA release. This is the first report about the influence of enalapril maleate on the CA secretion from the perfused model of the rat adrenal medulla.

II. MATERIALS AND 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 (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by 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 by saline-soaked gauge pads and urine in 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 entrance of 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 adrenal cortex. Then the adrenal gland, along with 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 ^{o}C (Fig. 1).

Perfusion of adrenal gland

The adrenal glands were perfused by means of 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; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95 % O_2 + 5 % CO_2 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 AngII (10^{-7} M) and DMPP (10^{-4} M) for 1 minutes and/or a single injection of ACh (5.32 x 10^{-3} M) and KCl (5.6 x 10^{-2} M) in a volume of 0.05 ml were made into perfusion stream via a three-way stopcock, respectively. McN-A-343 (10^{-4} M), veratridine (10^{-4} M), Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) 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 AngII, ACh, KCl, McN-A-343, veratridine, 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 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 enalapril or captopril on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing enalapril or captopril for 90 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 enalapril or captopril, 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

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981) using 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 stimulated glands by secretagogues used in the present work was high enough to obtain readings several folds greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of 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 pretreated 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 by computer program described by Tallarida and Murray (1987).

Drugs and their sources

The following drugs were used: enalapril maleate, captopril, cyclopiazonic acid, losartan, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1,4-dihydro-2, 6-dimethyl-3-nitro-4-(2 -trifluoro- methyl-phenyl)-pyridine-5 -carboxylate (BAY-K8644), angiotensin II acetate salt, 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 molar base.

III. RESULTS

Effects of enalapril on the 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=9). Since The *in vivo* effects of the ACE inhibitors (enalapril and captopril) on the adrenal medulla were first investigated by Critchley et al. (1988) and MacLean and Ungar (1986) who showed that they inhibited the CA release during splanchnic nerve stimulation of the dog adrenal gland. it was attempted initially to examine the effects of enalapril itself on the CA secretion from the perfused model of the rat adrenal glands. However, in the present study, enalapril (50 ~ 500 μM) 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 enalapril on the CA secretory responses evoked by AngII as well as by cholinergic receptor stimulation and direct membrane-depolarization. Secretagogues were given at 15~20 min-intervals. Enalapril 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 1169±41 ng for 4 min. However, when the concentration of enalapril was raised to the range of 50 ~ 500 μM for 90 min, there was a concentration-dependent decrease in ACh-evoked CA secretion. As shown in Fig. 2, in the presence of enalapril, CA releasing responses were inhibited by ~55% of the corresponding control release. Also, the depolarizing

agent, high potassium markedly stimulated the CA secretion (602±30 ng for 0-4 min). However, following the pretreatment with enalapril (50 ~ 500 μM), high K^+ (56 mM)-stimulated CA secretion was significantly inhibited by ~56% of the control at 75-94 min period as shown in Fig. 3. DMPP (100 μM), which is a selective nicotinic (N_N) receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (1218±52 ng for 0-8 min). However, as shown in Fig. 4, DMPP-evoked CA secretion after pretreatment with enalapril was reduced maximally to 55% of the control release (100%). McN-A-343 (100 μM), which is a selective muscarinic M_1-receptor agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 4 min also caused an increased CA secretion (539±23 ng for 0-4 min). However, in the presence of enalapril, McN-A-343-evoked CA secretion was markedly depressed to 57% of the corresponding control secretion (100%) as depicted in Fig. 5.

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

Since Bay-K-8644 is known to be a calcium channel activator, which enhances basal Ca^{2+} uptake (Garcia et al., 1984) and CA release (Lim et al., 1992), it was of interest to determine the effect of enalapril on Bay-K-8644-evoked CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10^{-5} M)-evoked CA secretion in the presence of enalapril (150 μM) was greatly blocked to 74% of the control at 60-94 min period as compared to the corresponding control release (496±16 ng for 0-4 min) from 8 adrenal glands as

shown in Fig. 6.

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger and Riley, 1989; Seidler et al., 1989). The inhibitory action of enalapril on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 7. In the presence of enalapril from 8 adrenal glands, cyclopiazonic acid (10 μM)-evoked CA secretion was also inhibited to 76% of the control response (486±16 ng for 0-4 min).

The voltage-dependent Na^+ channels consist of the principal α-subunit, which is associated with a noncovalently attached β_1-subunits, and a disulfide-linked β_2-subunit (Catterall, 2000). It has also been known that veratridine-induced Na^+ influx 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 (Wada et al., 1985). To characterize the pharmacological action of enalapril on voltage-dependent Na^+ channels, the effect of enalapril on the CA secretion induced by veratridine was examined here. As shown in Fig. 8, veratridine greatly produced CA secretion (1134±44 ng for 0-4 min). However, in the presence of enalapril (150 μM), veratridine (100 μM)-evoked CA secretion was greatly inhibited maximally to 60% of the corresponding control release at 75-94 min period.

Since it has been demonstrated that enalapril maleate, which is an ACE inhibitor, is used as an antihypertensive drug and blocks AngI conversion to AngII by its action on ACE (Murat and Ecemis, 1995; Katzung, 1995; Birincioglu et al., 1997), it was likely interesting to examine the effect of enalapril on AngII-evoked CA release. AngII (100 ηM) significantly evoked the CA secretory response

(576±24 ng for 0-4 min), whereas, in the presence of enalapril (150 μM), AngII (100 ηM)-evoked CA secretion was greatly inhibited to 50% of the corresponding control release (Fig. 9).

Influence of captopril on the CA secretion evoked by ACh, excess K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

As shown in Fig. 2~9, enalapril inhibited the CA secretory response evoked by cholinergic stimulation in the perfused rat adrenal gland. It has been shown that the adrenal CA secretion in response to hemorrhage was attenuated by captopril (an ACE inhibitor) in anesthetized cats (Feuerstein and Cohen, 1979) and dogs (Kimura et al., 1992). Therefore, in order to compare the effect of captopril with that of enalapril, it was likely of interest to examine effect of captopril on the CA secretion evoked by ACh, high K^+, DMPP and McN-A-343 from the isolated perfused rat adrenal glands. In the present study here, captopril (150 μM) itself also did not affect basal CA output from perfused rat adrenal glands (data not shown). In subsequent experiments, captopril (150 μM) was loaded into the rat adrenal medulla for 90 min immediately after establishment of control responses to cholinergic receptor-stimulation as well as direct membrane-depolarization. ACh (5.32 mM)-evoked CA release before perfusion with captopril (150 μM) was 1296±29 ng (0-4 min) from 8 rat adrenal glands. However, in the presence of captopril (150 μM) for 90 min, it was significantly attenuated to 68% of the control release (Fig. 10). High K^+ (56 mM)-evoked CA release in the presence of captopril (150 μM) was also reduced to 67% of the corresponding control secretion (768±24 ng, 0-4 min) from 8 glands, as shown in Fig. 11. In 7 rat

adrenal glands, DMPP (100 μM) perfused into the adrenal gland produced great CA secretion (1316±24 ng, 0-8 min) prior to loading with captopril. Following perfusion with captopril (150 μM) it was diminished to 68% of the corresponding control release (Fig. 12). Moreover, in the presence of captopril (150 μM), McN-A-343 (100 μM)-evoked CA secretory responses was also time-dependently inhibited by 71% of the control secretion (608±21 ng, 0-4 min) from 8 glands, as shown in Fig. 13.

Influence of captopril on the CA secretion evoked by Bay-K-8644, cyclopiazonic acid, veratridine and angiotensin II from the perfused rat adrenal glands

In the presence of captopril (150 μM), the secretory responses evoked by Bay-K-8644 (10 μM) and cyclopiazonic acid (10 μM) given into the adrenal gland for 4 min were greatly depressed to 71% and 74% of their the corresponding control responses (533±14 ng for 4 min and 491±14 ng for 4 min), respectively (Fig. 14 and 15). The CA secretion evoked by veratridine (100 μM), an activator of Na^+ channels, was greatly elevated to 1280±24 ng for 0-4 min before loading of captopril. However, in the presence of captopril, it was inhibited to 65% of the corresponding control secretion, as shown in Fig. 16. AngII (100 ηM) also markedly increased the CA secretory response (555±27 ng for 0-4 min), whereas in the presence of captopril (150 μM), AngII (100 ηM)-evoked CA secretion was greatly inhibited to 50% of the corresponding control release (Fig. 17).

Effects of enalapril plus losartan on CA release evoked by ACh, high

K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

As shown in Fig. 2~17, it was found here that both enalapril and captopril inhibit the CA secretory response evoked by cholinergic stimulation as well as direct membrane-depolarization in the perfused rat adrenal gland. Also, Noh and his co-workers (2009) have demonstrated that losartan (5~50 μM) dose-dependently inhibits the CA secretory responses evoked by ACh, high K^+, DMPP and McN-A-343 in the perfused model of rat adrenal glands. Therefore, to compare the combined effects of ACE inhibitor and AT_1-receptor antagonist on the adrenal CA release, the combined effects of enalapril and losartan on the CA secretion evoked by cholinergic receptor-stimulation as well as by membrane depolarization were examined here. In the simultaneous presence of enalapril (150 μM) and losartan (15 μM) for 90 min, ACh-evoked CA release was more reduced by 64~87% of the corresponding control release compared to those results of enalapril-treatment alone (71~89%) as illustrated in Fig. 18. High K^+ (56 mM)-evoked CA release in the simultaneous presence of enalapril (150 μM) and losartan (15 μM) for 90 min was also more inhibited by 63~90% of the corresponding control release during all periods in comparison to data of treatment with enalapril alone (74~97%) as shown in Fig. 19.

As shown in Fig. 20, during the simultaneous perfusion of enalapril and losartan for 90 min, the DMPP (100 μM)-evoked CA release more reduced by 65~89% of the corresponeding control response in comparison to that of the enalapril-treatment alone (72~91%). Moreover, in the presence of enalapril (150 μM) and losartan (15 μM), the CA secretory response evoked by McN-A-343 (100 μM) was also more diminished to 62~91% of the corresponding control

release compared to results of the enalapril-treatment alone (76~94%), as shown in Fig. 21.

Effects of enalapril plus losartan on CA release evoked by BAY-K-8644, cyclopiazonic acid, veratridine and angiotensin II from the perfused rat adrenal glands

As shown in Fig. 22, the simultaneous perfusion of enalapril (150 μM) and losartan (15 μM) for 90 min made more inihibition of the CA release evoked by Bay-K-8644 (10 μM) by 67~96% of the corresponding control response compared to the results of enalapril-treatment alone (74~100%). After the simultaneous perfusion with enalapril and losartan, cyclopiazonic acid (10 μM)-evoked CA reslease was also more reduced by 67~96% of the control release in comparison to the results following the treatment with enalapril alone (76~100%) as shown in Fig. 23. As depicted in Fig. 24, in the simultaneous presence of enalapril and losartan, veratridine (100 μM) -evoked CA reslease was also reduced by 50~74% of the corresponding control release compared to the results of enalapril-treatment alone (60~74%). As depicted in Fig. 25, during the simultaneous perfusion of enalapril (150 μM) and losartan (15 μM) for 90 min, the CA release evoked by AngII (100 ηM) was more inhibited by 44~65% of the corresponding control response compared to the inhibitory result of enalapril-treatment alone (50~72%)

IV. DISCUSSION

The present data have demonstrated that enalapril (ACE inhibitor) can inhibit the CA secretion evoked by AngII as well as by cholinergic (both nicotininc and muscarinic receptors) stimulation and direct membrane-depolarization from the perfused rat adrenal medulla. This inhibitory effect of enalapril seems to be mediated by blocking the influx of Na^+ and Ca^{2+} ions through their channels into adrenomedullary chromaffin cells as well as by inhibiting the Ca^{2+} release from cytoplasmic store, which appear to be mediated by blockade of local modulation in the CA secretion by the rat adrenomedullary chromaffin cells.

In support of this idea, the *in vivo* effects of the ACE inhibitors (enapril and captopril) on the adrenal medulla were first investigated by Critchley et al. (1988) and MacLean and Ungar (1986) who showed that they inhibited the CA release during splanchnic nerve stimulation of the anesthetized dog adrenal gland. Critchley and his co-workers (2004) also showed that electrical stimulation resulted in frequency-dependent increases in CA release from the anaesthetized dog's adrenal gland, and AngII also increased the CA secretion. Both candesartan (an AT_1 antagonist) and ramipril (an ACE inhibitor) diminished direct nerve stimulation-induced CA release. AngII also promotes the CA release from the adrenal medulla (Foucart et al., 1991), and the increased secretion of adrenal CAs induced by AngI was attributed to a direct action of AngI on adrenal chromaffin cells in anesthetized cats (Peach 1971; Peach et al., 1971). In terms of these results, the findings of the present study that both enalapril and captopril inhibit the CA secretory responses evoked by ACh, high K^+, DMPP and

McN-A-343 in the perfused rat adrenal medulla seem to be associated with ACE inhibition in adrenochromaffin cells. Moreover, it has also been suggested that ACE inhibition has a sympatho-inhibitory effect to the cold pressor test in human hypertensives (Minatoguchi et al., 1992). One possible explanation is that enalapril reduces AngII formation thus decreasing the activation of release-enhancing AngII receptors on chromaffin cells of the prfused rat adrenal medulla. Similar findings were reported in anesthetized rats, where the increase in plasma epinephrine concentration observed during hypoglycemia was reduced by ACE inhibitor (Feuerstein et al., 1977). Likewise, in the present work, the result that both enalapril and captopril significantly reduced the CA release evoked by AngII in the perfused rat adrenal medulla looks to support above findings. This means that ACE inhibitors such as enalapril and captopril can inhibit the CA release evoked by cholinergic receptor stimulatuion as well as by direct membrabe-depolarization through reduction of adrenal AngII syntheis.

In contrast to these findings, ACE inhibitors have been found to increase cardiac noradrenaline and adrenaline levels in spontaneously hypertensive rats (SHR) (Raasch et al., 2001). It has also been shown that in SHRs, noradrenaline and adrenaline contents were doubled in the left ventricle by captopril, enalapril, or candesartan independently of hypotensive potency but not in liver or cortex. In parallel, cardiac MAO activity was reduced by all doses of captopril, enalapril, or candesartan (Raasch et al**.,** 2002). Furthermore, It has been found that blockade of AngII formation by captopril did not affect hypoglycemia-induced activation of adrenal preganglionic adrenal sympathetic nerve activity (SNA), indicating that the RAS in the brain and spinal cord do not modulate increases in SNA during

hypoglycemia (Muntzel et al., 2005). These reports are quite different from the present results that both enalapril and captopril greatly attenuated the CA secretory responses evoked by AngII as well as ACh, high K^+, DMPP and McN-A-343. This discrepancy seems to be mediated by difference between experimental animals or methods (*in vivo* or *in vitro*).

The nicotinic receptor is a neurotransmitter-gated cation-conducting ion channel that is opened by binding of agonists such as ACh and DMPP (McGehee and Role, 1995). The opening of this channel triggers Ca^{2+} uptake and secretion of CA from chromaffin cells (Wada et al., 1985). To characterize if the inhibition of DMPP-stimulated secretion by enalapril was due to an effect on the activity of the nicotinic receptor, the effect of captopril, another ACE inhibitor, on DMPP-stimulated CA secretion was examined. As shown in Fig. 12, treatment with captopril greatly reduced DMPP-evoked CA secretion, inhibiting by 68% of its control release. In support of this finding, it has been reported that intravenous administration to pithed Wistar rats of enalapril (1.0 mg/kg) reduced pressor responses to the nicotinic ganglion stimulant DMPP (300 μg/kg, i.v.) (Rechtman et al., 1986). Previously, Boura and his co-workers (1982) have also demonstrated that in the cat and rat captopril depresses cardiovascular responses to sympathetic postganglionic nerve activation, both in the presence and absence of exogenous angiotensin I by an action which is proximal to the terminal synapse. It is likely plausible that enalapril or captopril can activate a signal transduction pathway that is altering the activity of both nicotinic receptors and voltage-sensitive Na^+ channels.

Activation of such a pathway could result in elevated levels of Ca^{2+}, diacylglycerol, and inositol bisphosphate in the cells. Consequently

Ca^{2+}-dependent and protein kinase C-dependent pathways may be activated. Protein kinase C has been reported to attenuate the activity of both nicotinic receptors (Swope et al., 1992) and voltage-sensitive Na^{+} channels (Catterall, 1992).

In the present study, both enalapril and captopril inhibited the CA secretory responses by high potassium, a direct membrane depolarizer, as well as by Bay-K-8644, an activator of L-type Ca^{2+} channels, which facilitates the influx of Ca^{2+} into the cells. This result indicates that both enalapril and captopril may inhibit Ca^{2+} influx into the rat adrenomedullary cells. Previously, Qi and his colleagues (1996) have shown that enalapril and nifedipine similarly decreased KCl-, NE-, and AngII-increased intracellular Ca^{2+} ($[Ca^{2+}]_i$) in cultured aortic smooth muscle cells (ASMC) of SHR. Captopril has also been reported to block KCl-, NE-, and AngII-increased $[Ca^{2+}]_i$ via a voltage-dependent Ca^{2+} channel of which function and specificity was altered in cultured ASMC of SHR (Qi et al., 1996). It is unclear how the ACE inhibition results in the inhibition of secretion seen in adrenal chromaffin cells. The simplest interpretation is that the decrease in Ca^{2+} uptake by enalapril or captoprilis 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 Ca^{2+} levels. Both the intracellular location of the Ca^{2+} level increase (Cheek, 1989; Ghosh and Greenberg, 1995) and the magnitude of the Ca^{2+} level increase (Holz et al., 1982) can affect the relationship between intracellular free Ca^{2+} levels and secretion. Holz et al. (1982) have reported that, when Ca^{2+} uptake is large, changes in Ca^{2+} uptake resulted in less than proportional changes in the CA secretion. Consequently, although the decrease in Ca^{2+} uptake (influx) into

the adrenal chromaffin cells may explain enalapril- or captopril-induced decrease in the CA secretion, it is still unclear whether this is only or even most important factor contributing to the inhibition of CA secretion by the ACE inhibitors. However, in view of the results so far obtained from the present study, it is felt that the voltage-sensitive Ca^{2+} channels located on chromaffin cell membrane of the rat adrenal medulla could be the target site for enalapril- or captopril-mediated inhibition of CA secretion. In more support of this idea, in cultured bovine adrenal medullary cells, nicotinic (but not muscarinic) receptors mediate the Ca^{2+}-dependent CA secretion (Fisher et al., 1981). It has also been known that the activation of nicotinic receptors stimulates the CA secretion by increasing Ca^{2+} entry through receptor-linked and/or voltage-dependent Ca^{2+} channels in both perfused rat adrenal glands (Wakade and Wakade, 1983; Lim and Hwang, 1991) and isolated bovine adrenal chromaffin cells (Kilpatrick et al, 1981; 1982; Knight and Kesteven, 1983). Wada and his coworkers (1985) have found that the adrenomedullary chromaffin cells have (i) nicotinic receptor-associated ionic channels, responsible for carbachol-induced Na^{+} influx, (ii) voltage-dependent Na^{+} channels, responsible for veratridine-induced Na^{+} influx and (iii) voltage-dependent Ca^{2+} channels, suggesting that the influx of Na^{+} caused either by carbachol or by veratridine leads to activate voltage-dependent Ca^{2+} channels by altering membrane potentials, whereas high K^{+} directly activates voltage-dependent Ca^{2+} channels without increasing Na^{+} influx. Therefore, in the present work, it seems that these inhibitory effects of both enalapril and captopril on the CA secretion evoked by DMPP and veratridine may be mediated by inhibiting Ca^{2+} influx through voltage-dependent Ca^{2+} channels due to activation of nicotinic receptor-associated ionic channels, responsible for

carbachol-induced Na^+ influx, as well as of voltage-dependent Na^+ channels, responsible for veratridine-induced Na^+ influx.

In the present study, both enalapril and captopril also inhibited the CA secretory responses evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger and Riley, 1989; Seidler et al., 1989). Therefore, it is felt that the inhibitory effect of enalapril or captoprilon the CA secretion evoked by cholinergic stimulation as well as by membrane-depolarization may be associated with the mobilization of intracellular Ca^{2+} in the chromaffin cells. This indicates that the ACE inhibitors cause an inhibitory effect on the release of Ca^{2+} from the intracellular pools induced by stimulation of muscarinic ACh receptors, which is weakly responsible for the secretion of CA. In the present work, enalapril or captopril significantly produced the inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests new other concept that enalapril or captopril 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 supporting 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 reticculum, which result in the increased subsequent Ca^{2+} release from those storage sites and thereby increases Ca^{2+}-dependent K^+-current (Suzuki et al., 1992). 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 (Cheek et al., 1989; Challiss et al., 1991). However, in

the present study, it is uncertain whether the inhibitory effect of the enalapril on Ca^{2+} movement from intracellular pools is due to their direct effect on the PI response or an indirect effect as a result of ACE inhibition by enalapril. It has been found that in bovine chromaffin cells, AngII causes Ca^{2+} entry via a pathway(s) activated as a consequence of internal store mobilization, and entry through this pathway(s) forms the majority of the sustained Ca^{2+} influx evoked by AngII (Powis et al., 2003). Based on previous results, the finding of the present work suggests that ACE inhibition may be involved in regulating CA secretion evoked by muscarinic M_1-receptor stimulation in the rat adrenal medullary chromaffin cells.

On the other hand, AT_1 receptor antagonist and ACE inhibitor have not been compared so far in respect to their effects on adrenal CA release. Thus, in the present work, these two classes of drugs have been compared the *in vitro* effects of an AT_1 receptor antagonist, losartan, and an ACE inhibitor, enalapril on the CA release evoked by AngII as wll as cholinergic stimulation and direct membrane depolarization, using the perfused model of the rat adrenal medulla. In the simultaneous presence of enalapril and losartan, the CA secretory responses evoked by AngII as well as by ACh, high K^+, DMPP and McN-A-343 were more markedly attenuated in comparison with the inhibitory level of enalapril- or losartan-treatment alone. Previously, during direct electrical stimulation of the anaesthetized dog's adrenal gland, both candesartan and ramipril reduced the CA release (Critchley et al., 2004). However, compared to basal release, the CA release during direct nerve stimulation was many times greater. Thus, circulating CA levels play a much less important role. Yamaguchi et al. (1999) have suggested that during direct stimulation of the gland AngII and other elements of

the RAS are released that may play a role in local regulation of the CA secretion. Hence, in the present study, it seems that losartan may act locally by inhibiting this regulation by blocking the AngII receptor on the chromaffin cell, while enalapril prevents local conversion of AngI to its active form. Thus, both drugs may act by inhibiting local RAS modulation. These two drugs also have an additive effect as the reduction in the CA release following their administration more decreased. This additive effect may be of benefit when treating conditions such as hypertension or heart failure. Both losartan and enalapril had similar outcomes on the CA release evoked by AngII as well as by cholinergic stimulation and direct membrane-depolarization. Similarly, Critchley and his co-workers (2004) showed that, when both candesartan and ramipril in the anaesthetized dog's adrenal gland were combined, their inhibitory effect catecholamine release was slightly enhanced. Also, AngII type-1 receptor bloker (ARB) + ACE inhibitor prevents myocardial fibrosis and decreases left ventriculat stiffness during the progression of congestive heart failure compared with ARB or ACE inhibitor alone (Funabiki et al., 2004). In one study, performed in spontaneously hypertensive rats, did a low-dose combination of enalapril and losartan exert synergistic reductions in blood pressure and left ventricular mass compared to adequate monotherapies (Menard et al., 1997). However, ACE inhibitors, such as enalapril, because they share a common pathway are known to cause bradykinin accumulation, which has the potential to cause cough (Koji et al., 2003). Thus, losartan may be advantageous over enalapril because it does not cause cough.

Previously, it has been shown that AT_1 receptor stimulation is most important as a regulatory factor for adrenomedullary CA synthesis and release. First, AT_1 blockade is sufficient to inhibit *in vivo* adrenal CA release by Ang II (Wong et al.,

1990). Second, pretreatment with an insurmountable AT_1 antagonist almost completely abolished the hormonal and sympathoadrenal response to the stress of isolation in unfamiliar metabolic cages (Armando et al., 2001). Third, losartan, an AT_1 antagonist, 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, and this inhibition seemed to be due to the blockade of AT_1 receptors located presynaptically on rat adrenomedullary chromaffin cells (Noh et al., 2009).

Taken together, as shown in Fig. 26, these experimental results suggest that both enalapril and captopril inhibit the CA secretion evoked by AngII as well as cholinergic stimulation (both nicotininc and muscarinic receptors) and direct membrane depolarization from the perfused rat adrenal medulla. It seems that both enalapril and captopril have antagonist activity at nicotinic receptors located on the adrenal chromaffin cells. It is also thought that this inhibitory effect of both enalapril and captopril may be mediated by blocking the influx of both Na^+ and Ca^{2+} 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 local ACE inhibtion. Based on these present data, it is suggested that the ACE may be involved in the perfused rat adrenomedullary CA secretion, and the combined use of ARB and ACE inhibitor has the beneficial advantage of controlling cardiovascular diseases, such as hypertension, congestive failure and renal failure, compared with ARB or ACE inhibitor alone.

V. SUMMARY

The *in vivo* effects of the angiotensin converting enzyme inhibitors (enalapril and captopril) on the adrenal medulla were first investigated by Critchley et al. (1988) and MacLean and Ungar (1986) who showed that they inhibited the release of catecholamines (CA) during splanchnic nerve stimulation of the dog adrenal gland. Armando and his colleagues (2004) demonstrated that both adrenomedullary AT_1 and AT_2 receptor types maintain and promote the adrenomedullary catecholamine synthesis and the transcriptional regulation of TH in rats. On the other hand, it has been found that the selective AT_1-receptor antagonist candesartan blocked $[Ca^{2+}]_i$ increase by Ang II in human adrenal chromaffin cells, while the receptor-AT_2 agonist, T_2-(Ang II 4–8)$_2$ was ineffective (Cavadas et al, 2003). Thus, there seems to be some controversy about the effects of ACE inhibitor and AT_1 receptor blocker on secretion of CA from the adrenal gland. The aim of the present study therefore was to examine whether ACE inhibitor can affect the CA release from the perfused model of the isolated rat adrenal gland, and also to compare the *in vitro* combined effects of an AT_1 receptor antagonist, losartan, and an ACE inhibitor, enalapril maleate, on adrenal CA release.

Enalapril (50~500 μM), perfused into an adrenal vein for 90 min, produced dose- and time-dependently inhibited 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). Furthermore, in adrenal glands loaded with enalapril (150 μM), the CA secretory responses evoked by Bay-K-8644 (10 μM, an

activator of L-type Ca^{2+} channels), cyclopiazonic acid (10 μM, an inhibitor of cytoplasmic Ca^{2+}-ATPase), veratridine (100 μM, an activator of voltage-dependent Na^{+} channels), and angiotensin II were markedly inhibited. Also, captopril (150 μM), an ACE inhibitor elicited similar inhibitory effect on above secretagogues-evoked CA secretion in time-dependent fashion. Both enalapril and captopril themselves did not affect basal CA output. When enalapril (150 μM) and losartan (15 μM) were combined, their inhibitory effect was slightly enhanced in comparison with that of enalapril (150 μM)- or losartan (15 μM)-treatment alone.

Collectively, these experimental results suggest that both enalapril and captopril inhibit the CA secretion evoked by AngII as well as cholinergic stimulation (both nicotininc and muscarinic receptors) and direct membrane depolarization from the perfused rat adrenal medulla. It seems that both enalapril and captopril have antagonist activity at nicotinic receptors located on the adrenal chromaffin cells. It is also thought that this inhibitory effect of both enalapril and captopril may be mediated by blocking the influx of both Na^{+} and Ca^{2+} through their voltage-dependent 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 local ACE inhibtion. Based on these present data, it is suggested that the ACE may be involved in the perfused rat adrenomedullary CA secretion, and the combined use of ARB and ACE inhibitor has the beneficial advantage of controlling cardiovascular diseases, such as hypertension, congestive failure and renal failure, compared with ARB or ACE inhibitor alone.

REFERENCES

Anton AH, Sayre DF. A study of the factors affecting the aluminum oxidetrihydroxy indole procedure for the analysis of catecholamines. *J Pharmacol Exp Ther* 138: 360-375, 1962.

Armando I, Carranza A, Nishimura Y, Hoe K-L, Barontini M, Terron JA, Falcon-Neri A, Ito T, Jourio AV, Saavedra JM. Peripheral administration of and angiotensin II AT_1 receptor antagonist decreases the hypothalamic-pituitary-adrenal response to isolation stress. *Endocrinology* 142: 3880–3889, 2001.

Armando I, Jezova M, Bregonzio C, Baiardi G, Saavedra JM. Angiotensin II AT1 and AT2 receptor types regulate basal and stress-induced adrenomedullary catecholamine production through transcriptional regulation of tyrosine hydroxylase. *Ann N Y Acad Sci* 1018: 302-309, 2004.

Birincioglu M, Aksoy T, Olmez E, Acet A. Protective effect of ACE inhibitors on ischemia-reperfusion-induced arrhythmias in rats: is this effect related to the free radical scavenging action of these drugs? *Free Rad Res* 27: 389-396, 1997.

Boura AL, Hui SC, Rechtman MP, Walters WA. Captopril inhibits pressor responses to peripheral sympathetic nerve activation in cats and rats. *Clin Exp Pharmacol Physiol* 7: 123-128, 1982.

Catterall WA. Cellular and molecular biology of voltage-gated sodium channels. *Physiol Rev* 72(4 Suppl): S15-48, 1992.

Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron* 26(1): 13-25, 2000.

Cavadas C, Grand D, Mosimann F, Cotrim MD, Fontes Ribeiro CA, Brunner HR, Grouzmann E., Angiotensin II mediates catecholamine and neuropeptide Y secretion in human adrenal chromaffin cells through the AT1 receptor. *Regul Pept* 111(1-3): 61-65, 2003.

Chai SY, Allen AM, Adam WR, Mendelsohn FAO. Local actions of angiotensin II: quantitative in vitro autoradiographic localization of angiotensin II receptor binding and angiotensin converting enzyme in target tissues. *J Cardiovasc Pharmacol* 8(Suppl. 10): S35–S39, 1986.

Challiss RA, Jones JA, Owen PJ, Boarder MR. Changes in inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate mass accumulations in cultured adrenal chromaffin cells in response to bradykinin and histamine. *J Neurochem* 56: 1083-1086, 1991.

Cheek TR, O'Sullivan AJ, Moreton RB, Berridge MJ, Burgoyne RD. Spatial localization of the stimulus-induced rise in cyrosolic Ca^{2+} in bovine adrenal chromaffin cells: Distinct nicotinic and muscarinic patterns. *FEBS Lett* 247: 429-434, 1989.

Critchley JA, MacLean MR, Ungar A. Inhibitory regulation by co-released peptides of catecholamine secretion by the canine adrenal medulla. *Br J Pharmacol* 93: 383–386, 1988.

Critchley L, Ding B, Fok B, Wang D, Tomlinson B, James A, Thomas GN, Critchley J. The effects of candesartan and ramipril on adrenal

catecholamine release in anaesthetized dogs. *Eur J Pharmacol* 489: 67-75, 2004.

Feurerstein G, Boonyaviroj P, Gutman Y. Renin-angiotensin mediation of adrenal catecholamine secretion induced by haemorrhage. *Eur J Pharmacol* 44: 131-142, 1977.

Feuerstein G, Cohen S. Modification by SQ 14225 of blood pressure and adrenal catecholamine response to haemorrhage. *Eur J Pharmacol* 55: 203–206, 1979.

Fisher SK, Holz RW, Agranoff BW. Muscarinic receptors in chromaffin cell culture mediate enhanced phospholipid labeling but not catecholamine secretion. *J Neurochem* 37: 491-487, 1981.

Forster C, Le Tran Y. Angiotensin: vasodilator or antagonist. *Brit J Pharmacol* 117: 43, 1996.

Foucart S, de Champlain J, Nadeau R. Modulation by beta-adrenoceptors and angiotensin II receptors of splanchnic nerve evoked catecholamine release from the adrenal medulla. *Can J Physiol Pharm* 69: 1–7, 1991.

Funabiki K, Onishi K, Dohi K, Koji T, Imanaka-Yoshida K, Ito M, Wada H, Isaka N, Nobori T, Nakano T. Combined angiotensin receptor blocker and ACE inhibitor on myocardial fibrosis and left ventricular stiffness in dogs with heart failure. *Am J Physiol Heart Circ Physiol* 287: H2487-H2492, 2004.

Garcia AG, Sala F, Reig JA, Viniegra S, Frias J, Fonteriz R, Gandia L. Dihydropyridine Bay-K-8644 activates chromaffin cell calcium channels. *Nature* 309: 69-71, 1984.

Ghosh A, Greenberg ME. Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* 268: 239-247, 1995.

Giannattasio C, Cattaneo BM, Omboni S, et al. Sympathomoderating influence of benazepril in essential hypertension. *J Hypertens* 10: 373-378, 1992.

Goeger DE, Riley RT. Interaction of cyclopiazonic acid with rat skeletal muscle sarcoplasmic reticulum vesicles. Effect on Ca^{2+} binding and Ca^{2+} permeability. *Biochem Pharmacol* 38: 3995-4003, 1989.

Hammer R, Giachetti A. Muscarinic receptor subtypes: M_1 and M_2 biochemical and functional characterization. *Life Sci* 31: 2992-2998, 1982.

Holz RW, Senter RA, Frye RA. Relationship between Ca^{2+} uptake and catecholamine secretion in primary dissociated cultures of adrenal modulla. *J Neurochem* 39: 635-640, 1982.

Israel A, Barbella Y, Saavedra JM. Compensatory increase in adrenomedullary angiotensin-converting enzyme activity (kininase II) after unilateral adrenalectomy. *Regul Pept* 16: 97–105, 1986.

Katzung BG. Temel ve Klinik Farmakoloji Cilt I. *İstanbul: Barış Kitabevi* 1995.

Kilpatrick DL, Slepetis RJ, Corcoran JJ, Kirshner N. Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells. *J Neurochem* 38: 427-435, 1982.

Kilpatrick DL, Slepetis RJ, Kirshner N. Ion channels and membrane potential in stimulus-secretion coupling in adrenal medulla cells. *J Neurochem* 36: 1245-1255, 1981.

Kimura T, Suzuki Y, Yoneda H, Suzuki-Kusaba M, Satoh S. Facilitatory role of the renin–angiotensin system in controlling adrenal catecholamine release in hemorrhaged dogs. *J Cardiovasc Pharmacol* 19: 975–981, 1992.

Knight DE, Kesteven NT. Evoked transient intracellular free Ca^{2+} changes and secretion in isolated bovine adrenal medullary cells. *Proc R Soc Lond Biol*

Sci 218: 177-199, 1983.

Koji T, Onishi K, Dohi K, Okamoto R, Tanabe M, Kitamura T, Ito M, Isaka N, Nobori T, Nakano T. Addition of angiotensin ii receptor antagonist to an ACE inhibitor in heart failure improves cardiovascular function by a bradykinin-mediated mechanism. *J Cardiovasc Pharmacol* 41: 632–639, 2003.

Le Tran Y, Forster C. Effect of angiotensin receptor blockade in the rabbit aorta: influence of the endothelium. *Can J Physiol Pharmacol* 74: 1277-1286, 1996.

Lim DY, Hwang DH. Studies on secretion of catecholamines evoked by DMPP and McN-A-343 in the rat adrenal gland. *Korean J Pharmacol* 27: 53-67, 1991.

Lim DY, Kim CD, Ahn KW. Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands. *Arch Pharm Res* 15: 115-125, 1992.

MacLean MR, Ungar A. Effects of the renin–angiotensin system on the reflex response of the adrenal medulla to hypotension in the dog. *J Physiol* 373: 343–352, 1986.

Martineau D, Lamouche S, Briand R, Yamaguchi N. Functional involvement of angiotensin AT2 receptor in adrenal catecholamine secretion in vivo. *Can J Physiol Pharmacol* 77: 367-374, 1999.

Martineau D, Yamaguchi N, Briand R. Inhibition by BMS 186295, a selective nonpeptide AT1 antagonist, of adrenal catecholamine release induced by angiotensin II in the dog in vivo. *Can J Physiol Pharmacol* 73: 459-464, 1995.

McGehee DS, Role LW. Physiological diversity of nicotinic acetylcholine

receptors expressed by vertebrate neurons. *Annu Rev Physiol* 57: 521-546, 1995.

McGrath BP, Arnolda LF. Enalapril reduces the catecholamine response to exercise in patients with heart failure. *Eur J Clin Pharmacol* 30: 485-487, 1986.

Menard J, Campbell DJ, Azizi M, Gonzales MF. Synergistic effects of ACE inhibition and Ang II antagonism on blood pressure, cardiac weight, and renin in spontaneously hypertensive rats. *Circulation* 96: 3072–3078, 1997.

Minatoguchi S, Ito H, Koshiji M, Masao K, Hirakawa S, Majewski H. Enalapril decreases plasma noradrenaline levels during the cold pressor test in human hypertensives. *Clin Exp Pharmacol Physiol* 19: 279-282, 1992.

Muntzel MS, Joseph T, Onwumere O. Captopril does not affect reflex increases in adrenal or lumbar sympathetic nerve activity to hypoglycemia in rats. *Metabolism* 54: 1679-1686, 2005.

Murat SN, Ecemiş ZA. Angiotensin konverting enzim inhibitörleri ve klinik kullanimi. *Türkiye Tıp Dergisi* 2: 172-184, 1995.

Nakamura M, Misono KS, Naruse M, Workman RJ, Inagami T. A role for the adrenal renin–angiotensin system in the regulation of potassium-stimulated aldosterone production. *Endocrinology* 117: 1772–1778, 1985.

Noh HJ, Kang YS, Lim DY. Effects of Losartan on Catecholamine Release in the Isolated Rat Adrenal Gland. *Korean J Physiol Pharmacol* 13: 327-335, 2009.

Oda H, Lotshaw DP, Franco-Saenz R, Mulrow PJ. Local generation of angiotensin II as a mechanism of aldosterone secretion in rat adrenal capsules. *Proc Soc Exp Biol Med* 196: 175–177, 1991.

Peach MJ. Adrenal medullary stimulation induced by angiotensin I, angiotensin II,

and analogues. *Circ Res* 28 and 29(Suppl. II): II107-116, 1971.

Peach MJ, Bumpus FM, Khairallah PA. Release of adrenal catecholamines by angiotensin I. *J Pharmacol Exp Ther* 176: 366–376, 1971.

Powis DA, Zerbes M, Herd LM, Dunkley PR. Angiotensin II causes calcium entry into bovine adrenal chromaffin cells via pathway(s) activated by depletion of intracellular calcium stores. *Neurochem* Res 28: 1299-1306, 2003.

Qi JH, Zhang L, Wang J, Wei PJ, Gu PK, Jin ZJ, Huang MZ, Wang HY. Effects of captopril and enalapril on intracellular Ca2+ in vascular smooth muscle cell. *Zhongguo Yao Li Xue Bao* 17: 142-145, 1996.

Raasch W, Bartels T, Gieselberg A, Dendorfer A, Dominiak P. Angiotensin I-converting enzyme inhibition increases cardiac catecholamine content and reduces monoamine oxidase activity via an angiotensin type 1 receptor-mediated mechanism. *J Pharmacol Exp Ther* 300: 428–434, 2002.

Raasch W, Betge S, Dendorfer A, Bartels T, Dominiak P. Angiotensin converting enzyme inhibition improves cardiac neuronal uptake of noradrenaline in spontaneously hypertensive rats. *J Hypertens* 19: 1827-1833, 2001.

Rechtman MP, Ishac EJ, Boura AL, King RG, Walters WA. Attenuation of pressor responses to sympathetic stimuli in the rat by enalapril. *Clin Exp Pharmacol Physiol* 13: 691-697, 1986.

Seidler NW, Jona I, Vegh N, Martonosi A. Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasimc reticulum. *J Biol Chem* 264: 17816-17823, 1989.

Strittmatter SM, De Souza EB, Lynch DR, Snyder SH. Angiotensin-converting enzyme localized in the rat pituitary and adrenal glands by [3H]captopril autoradiography. *Endocrinology* 118: 1690–1699, 1986.

Suzuki M, Muraki K, Imaizumi Y, Watanabe M. Cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-pump, reduces Ca^{2+}-dependent K^{+} currents in guinea-pig smooth muscle cells. *Br J Pharmacol* 107: 134-140, 1992.

Swope SL, Moss SJ, Blackstone CD, Huganir RL. Phosphorylation of ligand-gated ion channels: a possible mode of synaptic plasticity. *FASEB J* 6: 2514-2523, 1992.

Talas ZS, Yurekli M. The effects of enalapril maleate and cold stress exposure on tyrosine hydroxylase activity in some rat tissues. *Cell Biochem Funct* 24: 537-540, 2006.

Tallarida RJ, Murray RB. Manual of pharmacologic calculation with computer programs. 2nd ed. Speringer-Verlag, New York, p 132, 1987.

Wada A, Takara H, Izumi F, Kobayashi H, Yanagihara N. Influx of ^{22}Na through acetylcholine receptor-associated Na channels: relationship between ^{22}Na influx, ^{45}Ca influx and secretion of catecholamines in cultured bovine adrenal medulla cells. *Neuroscience* 15: 283-292, 1985.

Wakade AR. Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J Physiol* 313: 463-480, 1981.

Wakade AR, Wakade TD. Contribution of nicotinic and muscarinic receptors in the secretion of catecholamines evoked by endogenous and exogenous acetylcholine. *Neuroscience* 10: 973-978, 1983.

Wong PC, Hart SD, Zaspel AM, Chiu AT, Ardecky RJ, Smith RD, Timmermans PB. Functional studies of nonpeptide angiotensin II receptor subtype-specific ligands: DuP 753 (AII-1) and PD123177 (AII-2). *J Pharmacol Exp Ther* 255: 584–592, 1990.

Yamaguchi N, Martineau D, Lamouche S, Briand R. Functional role of local angiotensin-converting enzyme (ACE) in adrenal catecholamine secretion in vivo. *Can J Physiol Pharm* 77: 878–885, 1999.

Influence of ACE Inhibitor on Nicotinic Stimulation-Evoked Catecholamine Secretion in the Perfused Rat Adrenal Medulla

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The *in vivo* effects of the angiotensin converting enzyme inhibitors (enalapril and captopril) on the adrenal medulla were first investigated by Critchley et al. (1988) and MacLean and Ungar (1986) who showed that they inhibited the release of catecholamines (CA) during splanchnic nerve stimulation of the dog adrenal gland. Armando and his colleagues (2004) demonstrated that both adrenomedullary AT_1 and AT_2 receptor types maintain and promote the adrenomedullary catecholamine synthesis and the transcriptional regulation of TH in rats. On the other hand, it has been found that the selective AT_1-receptor antagonist candesartan blocked $[Ca^{2+}]_i$ increase by Ang II in human adrenal chromaffin cells, while the receptor-AT_2 agonist, T_2-(Ang II 4–8)$_2$ was ineffective (Cavadas et al, 2003). Thus, there seems to be some controversy about the effects of ACE inhibitor and AT_1 receptor blocker on secretion of CA from the adrenal gland. The aim of the present study therefore was to examine whether ACE inhibitor can affect the CA release from the perfused model of the isolated

rat adrenal gland, and also to compare the *in vitro* combined effects of an AT_1 receptor antagonist, losartan, and an ACE inhibitor, enalapril maleate, on adrenal CA release.

Enalapril (50~500 μM), perfused into an adrenal vein for 90 min, produced dose- and time-dependently inhibited 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). Furthermore, in adrenal glands loaded with enalapril (150 μM), the CA secretory responses evoked by Bay-K-8644 (10 μM, an activator of L-type Ca^{2+} channels), cyclopiazonic acid (10 μM, an inhibitor of cytoplasmic Ca^{2+}-ATPase), veratridine (100 μM, an activator of voltage-dependent Na^+ channels), and angiotensin II were markedly inhibited. Also, captopril (150 μM), an ACE inhibitor elicited similar inhibitory effect on above secretagogues-evoked CA secretion in time-dependent fashion. Both enalapril and captopril themselves did not affect basal CA output. When enalapril (150 μM) and losartan (15 μM) were combined, their inhibitory effect was slightly enhanced in comparison with that of enalapril (150 μM)- or losartan (15 μM)-treatment alone.

Collectively, these experimental results suggest that both enalapril and captopril inhibit the CA secretion evoked by AngII as well as cholinergic stimulation (both nicotininc and muscarinic receptors) and direct membrane depolarization from the perfused rat adrenal medulla. It seems that both enalapril and captopril have antagonist activity at nicotinic receptors located on the adrenal chromaffin cells. It is also thought that this inhibitory effect of both enalapril and captopril may be mediated by blocking the influx of both Na^+ and Ca^{2+} through

their voltage-dependent 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 local ACE inhibtion. Based on these present data, it is suggested that the ACE may be involved in the perfused rat adrenomedullary CA secretion, and the combined use of ARB and ACE inhibitor has the beneficial advantage of controlling cardiovascular diseases, such as hypertension, congestive failure and renal failure, compared with ARB or ACE inhibitor alone.

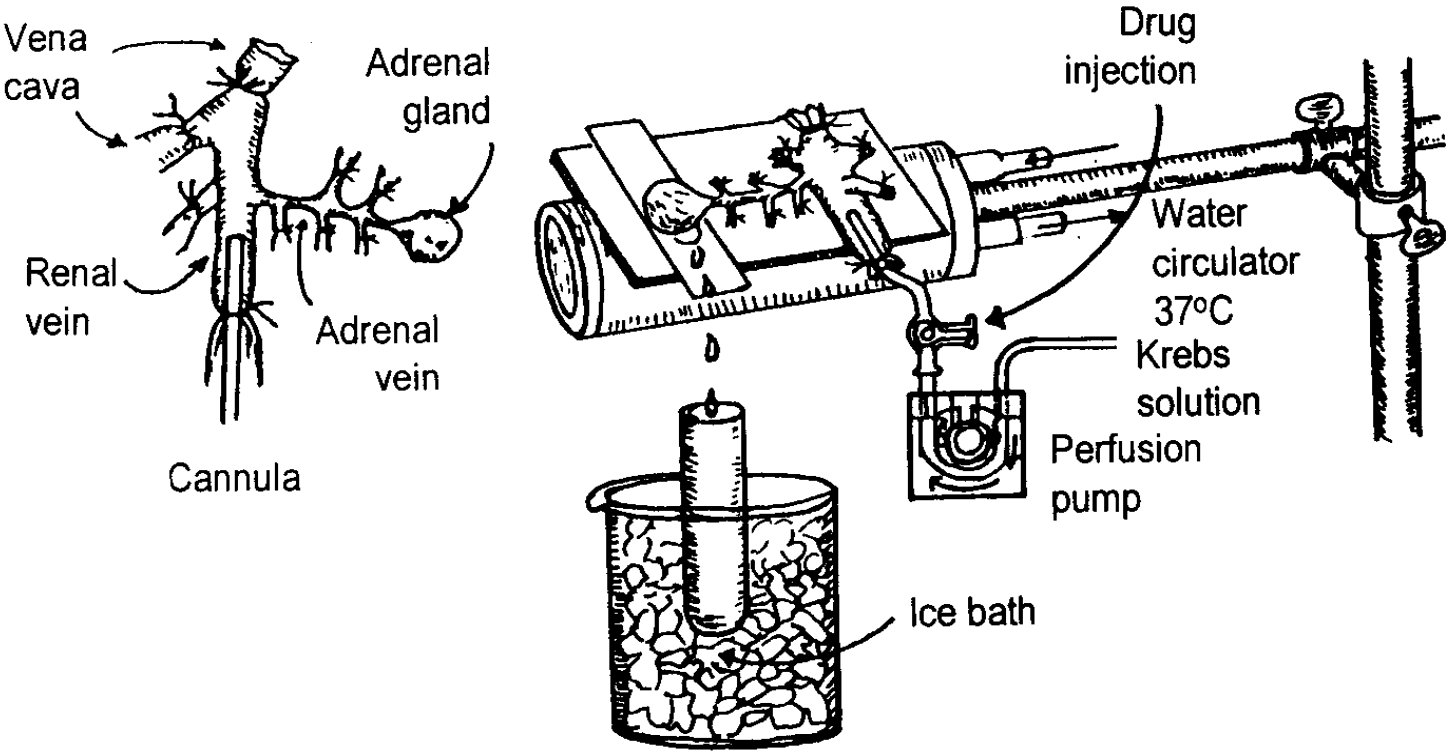


Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamines in the isolated perfused rat adrenal gland.

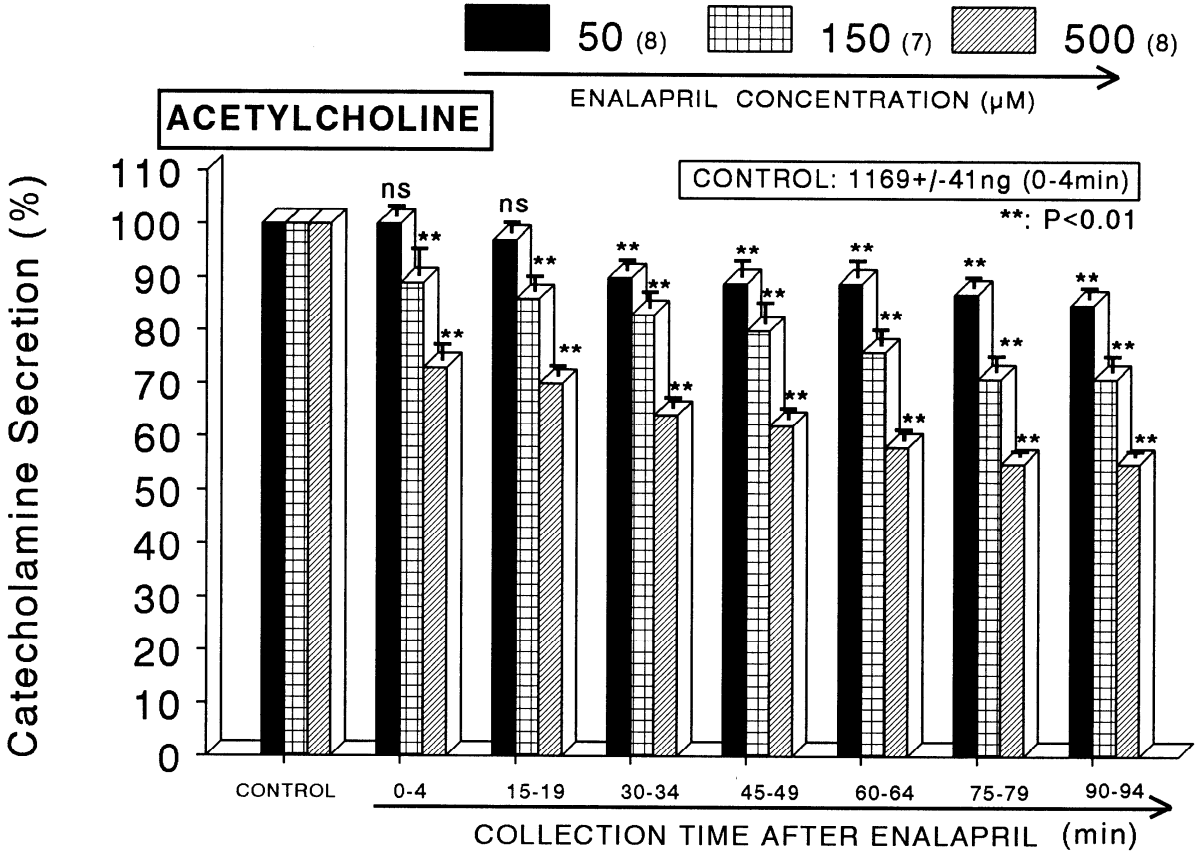


Fig. 2. Dose-dependent effect of enalapril on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. The CA secretion by a single injection of ACh (5.32 mM) in a volume of 0.05 ml was evoked at 15 min intervals for 90 min after loading with 50, 150, 500 μM of enalapril 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 for 4 min). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-pretreated group of enalapril. ACh-induced perfusate was collected for 4 minutes. **: $P < 0.01$. ns: Statistically not significant.

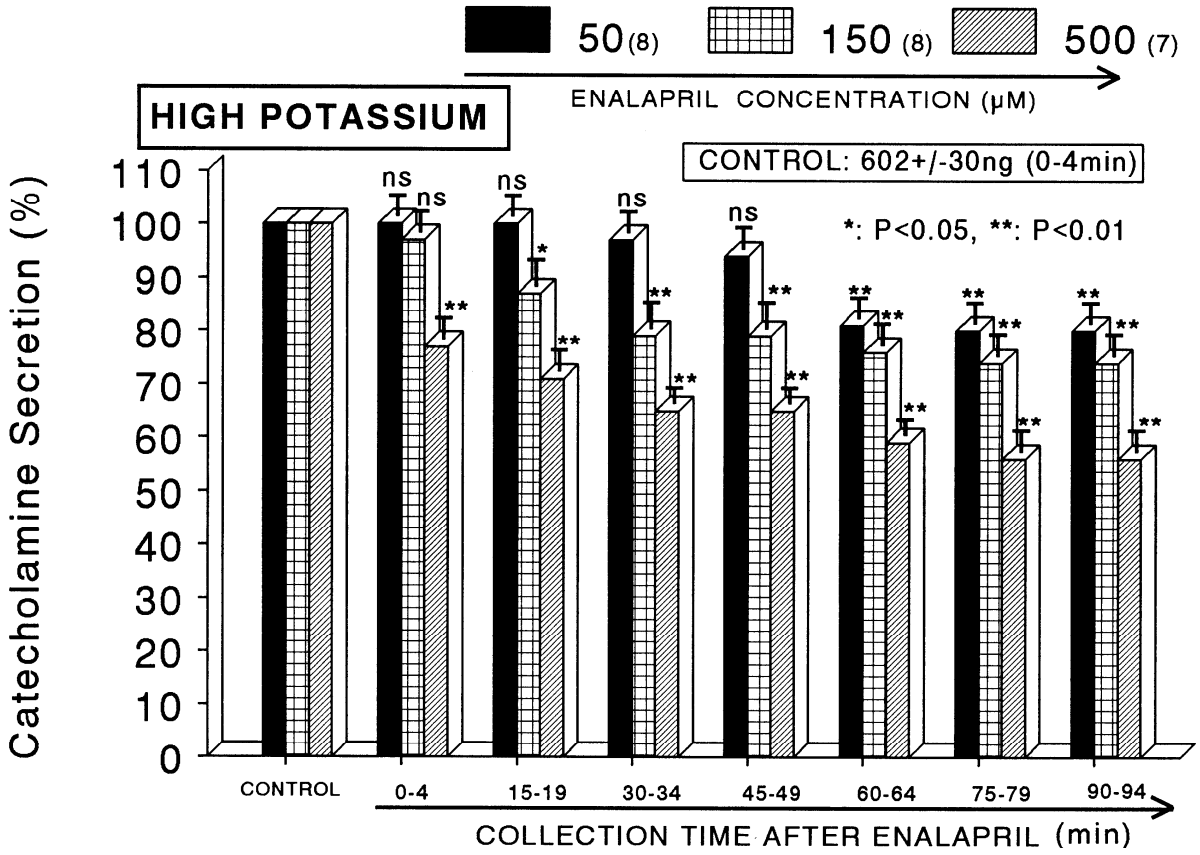


Fig. 3. Dose-dependent effect of enalapril on the secretory responses of catecholamines (CA) evoked by high K^+ from the isolated perfused rat adrenal glands. The single injection of K^+ (56 mM) in a volume of 0.1 ml was given at 15 min intervals for 90 min after loading with 50, 150, 500μM of enalapril, respectively. Statistical difference was obtained by comparing the corresponding control (CONTROL) with each concentration-pretreated group of enalapril. K^+-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

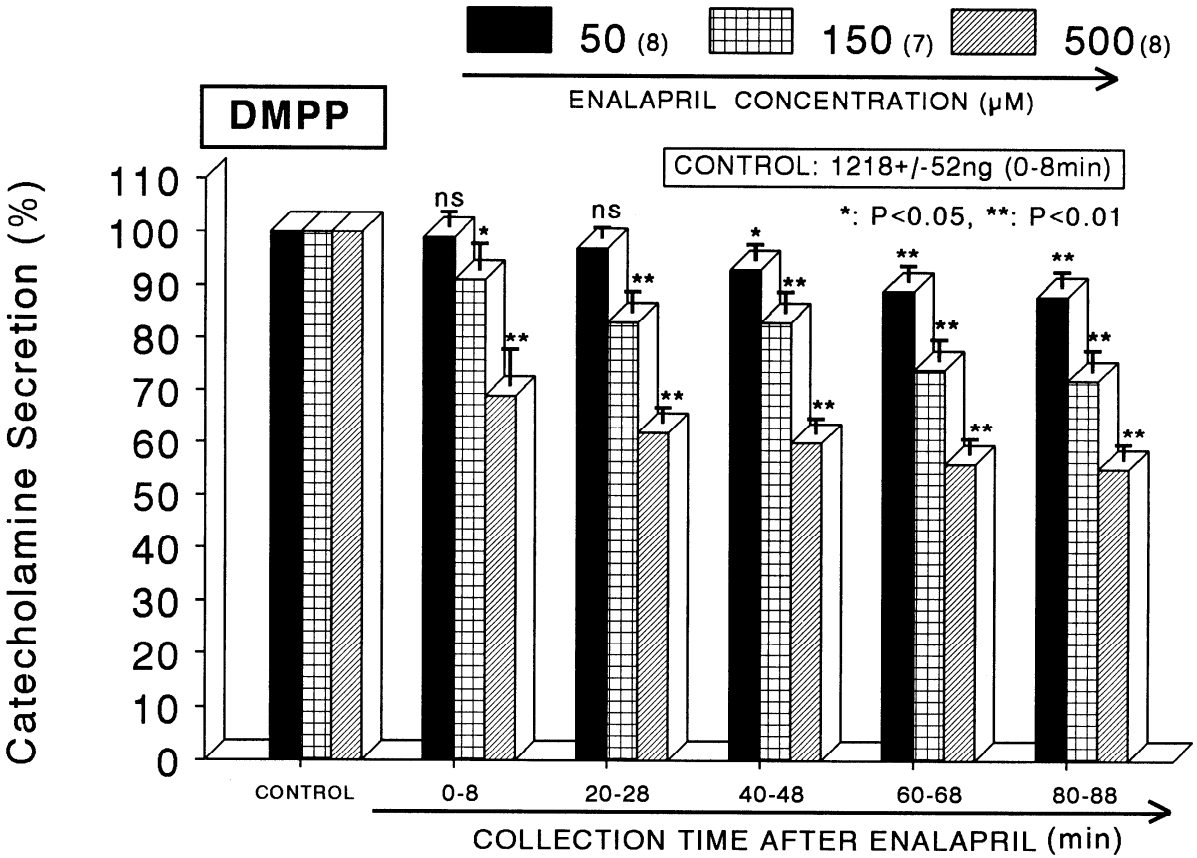


Fig. 4. Dose-dependent effect of enalapril on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. The CA secretion by the perfusion of DMPP (100 μM) for 1 min was evoked at 20 min intervals for 90 min after loading with 50, 150, 500μM of enalapril, respectively. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of enalapril. DMPP-induced perfusate was collected for 8 minutes. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

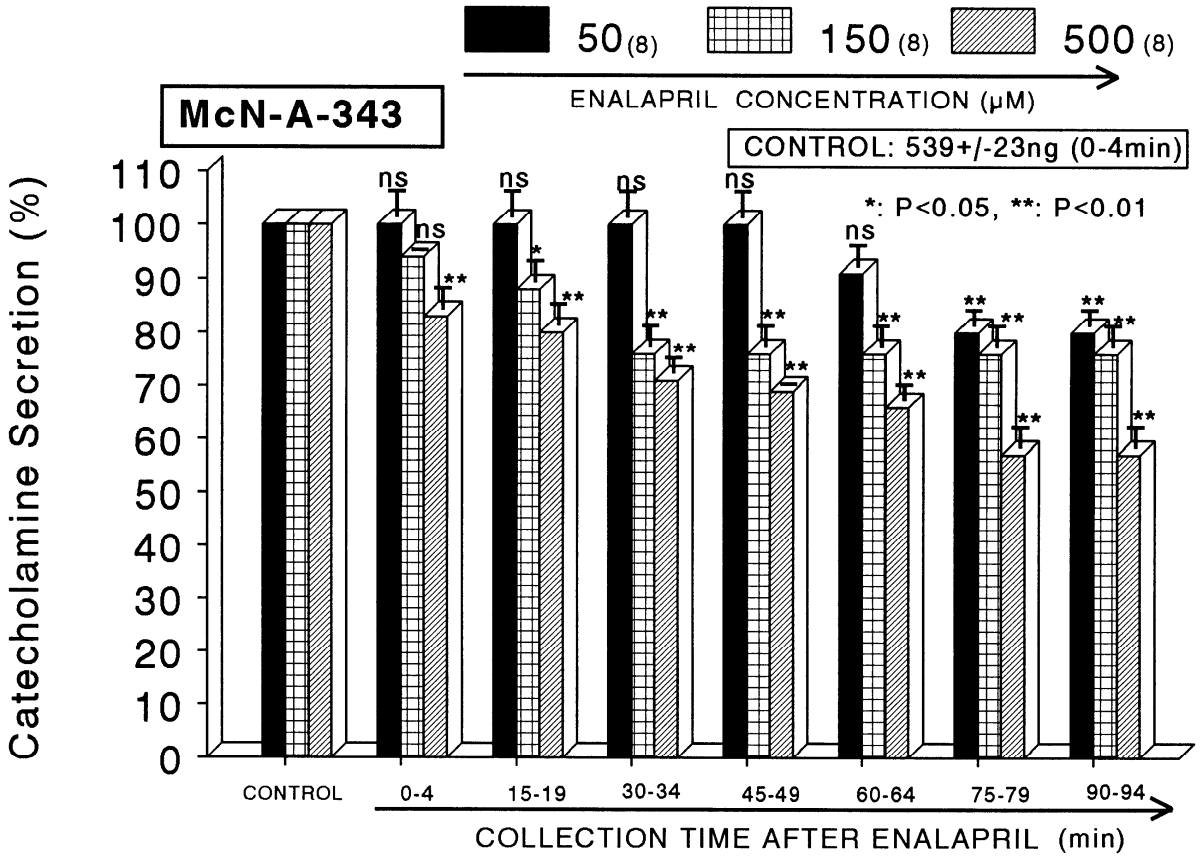


Fig. 5. Dose-dependent effect of enalapril on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. The CA secretion by the perfusion of McN-A-343 (100 μM) for 4 min was evoked at 15 min intervals for 90 min after loading with 50, 150, 500μM of enalapril, respectively. Statistical difference was obtained by comparing the corresponding control with each concentration-treated group of enalapril. McN-A-343-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 2. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

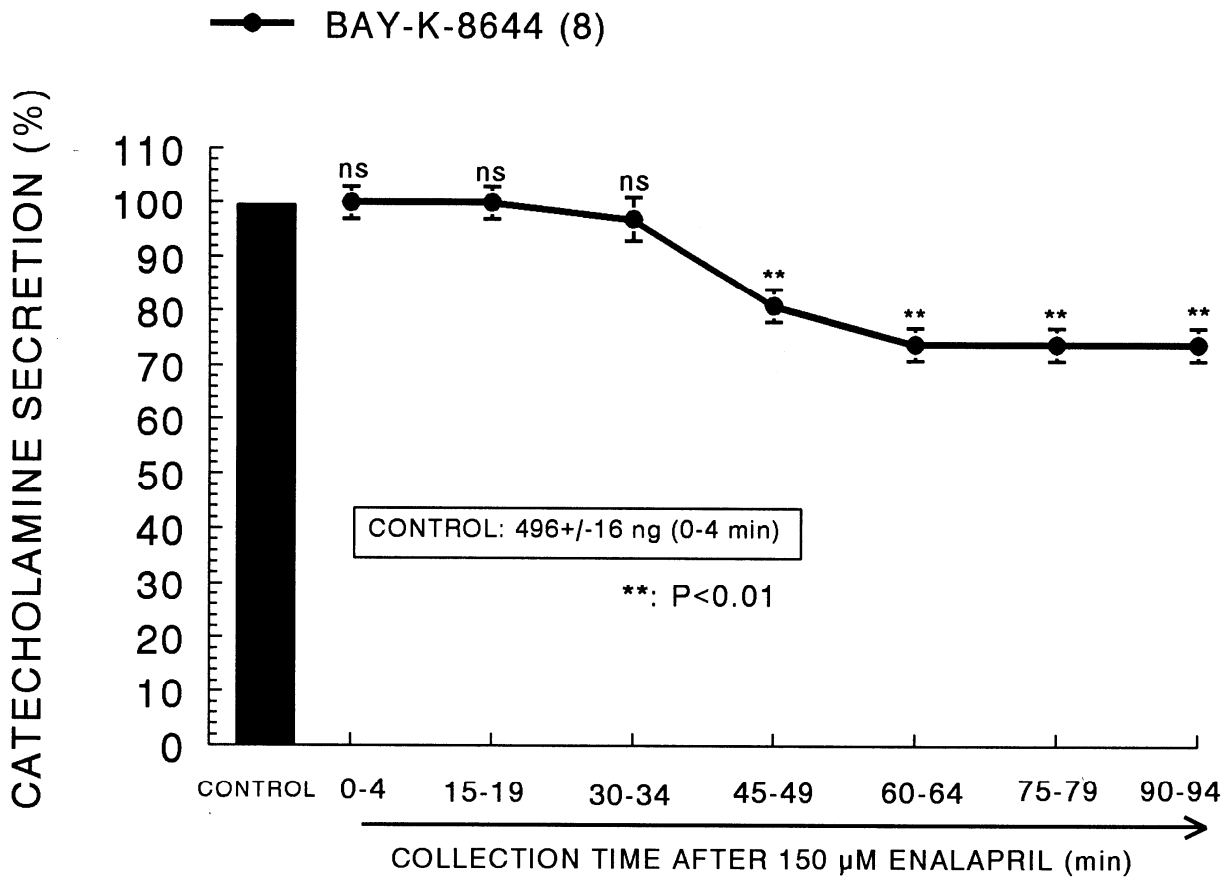


Fig. 6. Time-course effects of enalapril on CA release evoked by Bay-K-8644 from the perfused rat adrenal glands. Perfusion of Bay-K-8644 (10 μM) for 4 min was made at 15 min intervals for 90 min after loading with enalapril (150 μM). Statistical difference was obtained by comparing the corresponding control with each period after treatment with enalapril. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

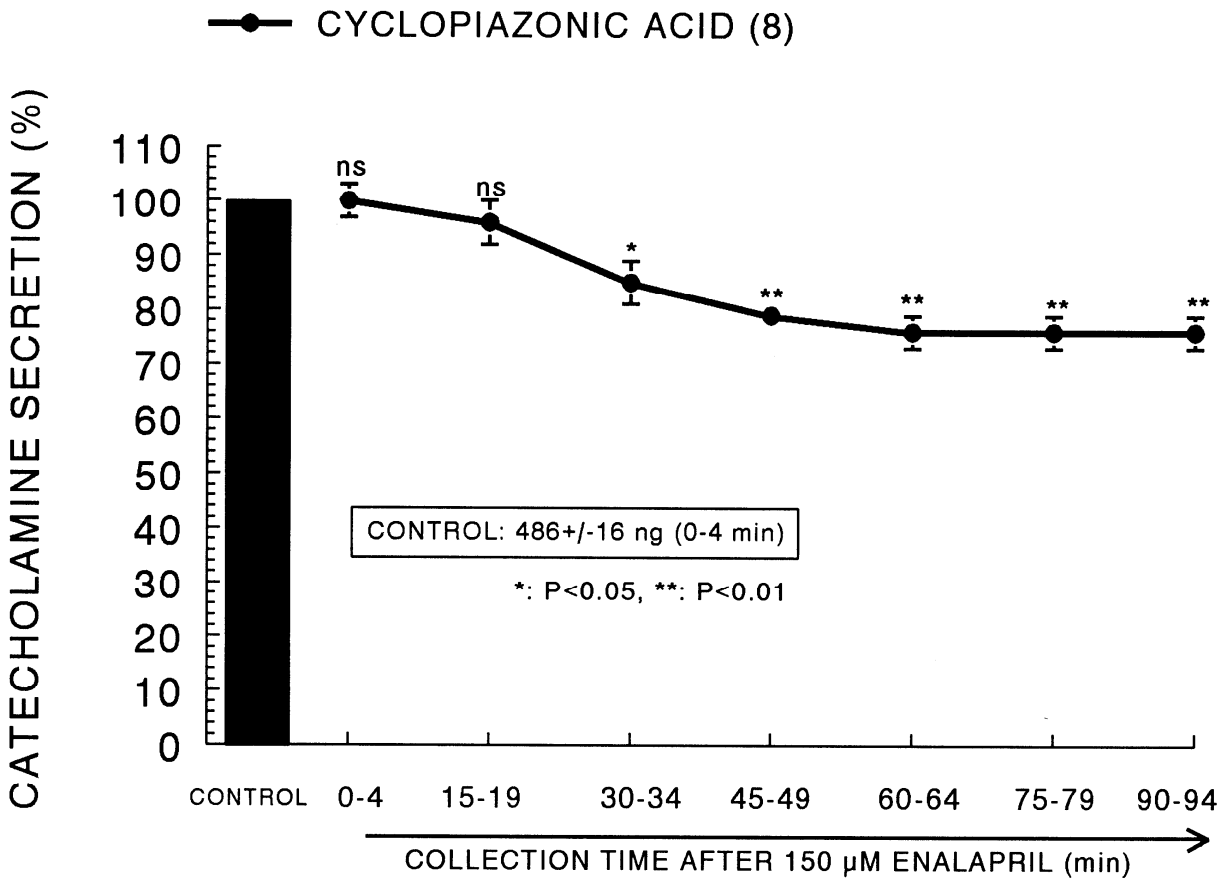


Fig. 7. Time-course effects of enalapril on CA release evoked by cyclopiazonic acid from the perfused rat adrenal glands. Perfusion of cyclopiazonic acid (10 μM) for 4 min was made at 15 min intervals for 90 min after loading with enalapril (150 μM). Statistical difference was obtained by comparing the corresponding control with each period after pretreatment with enalapril. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

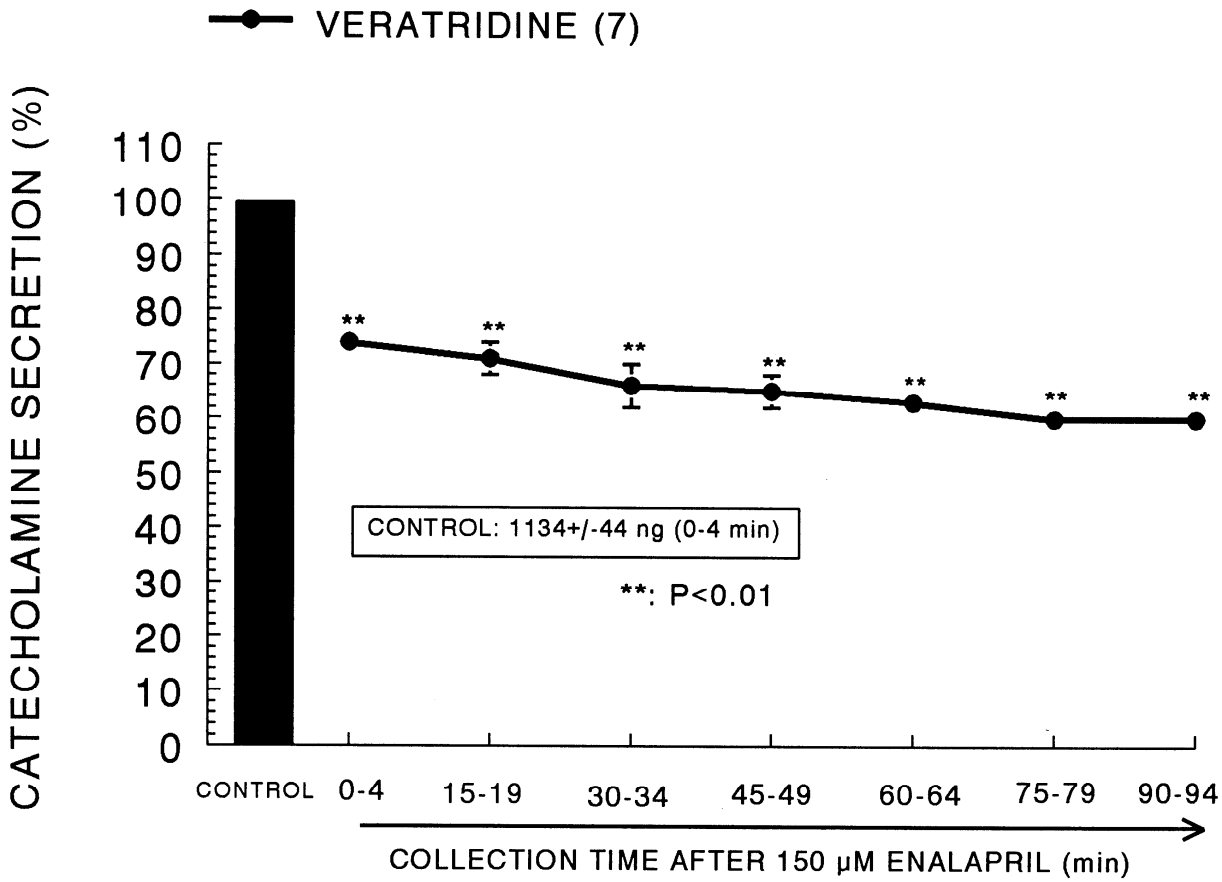


Fig. 8. Time-course effects of enalapril on the CA release evoked by veratridine from the perfused rat adrenal glands. Perfusion of veratridine (100 μM) for 4 min was made at 15 min intervals for 90 min after loading with enalapril (150 μM). Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

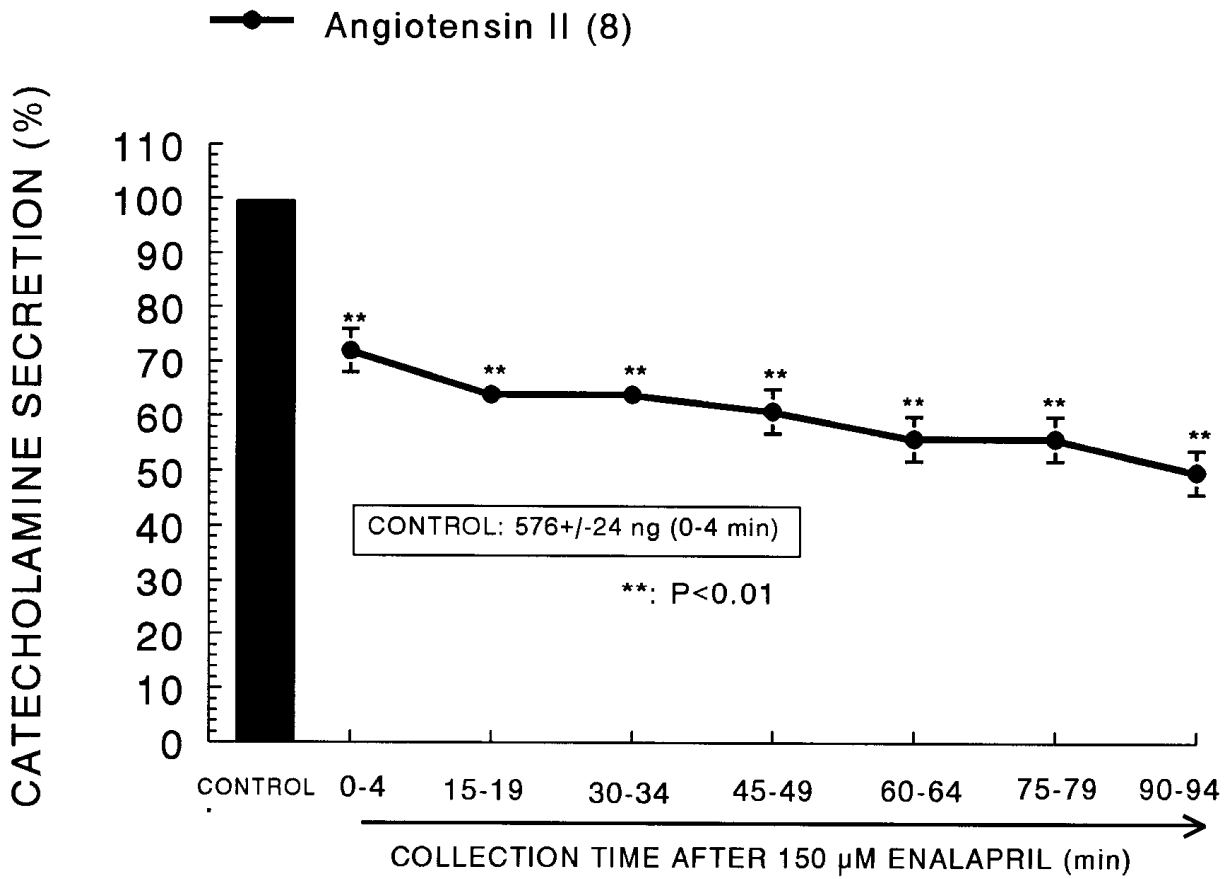


Fig. 9. Time-course effects of enalapril on the CA release evoked by angoitensin II from the perfused rat adrenal glands. Perfusion of angoitensin II (100 ηM) for 1 min was made at 15 min intervals for 90 min after loading with enalapril (150 μM). Other legends are the same as in Fig. 2. **: P < 0.01. ns: Statistically not significant.

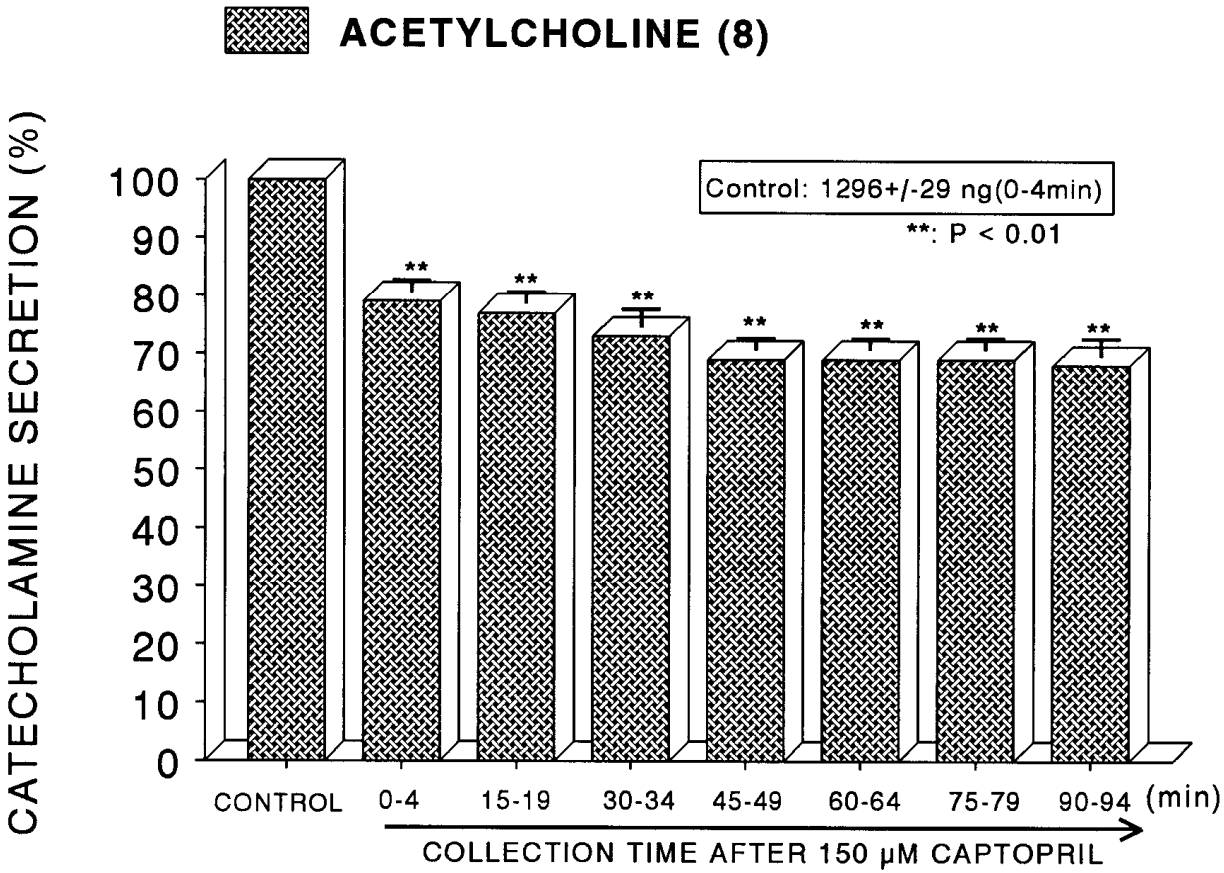


Fig. 10. Time-course effect of captopril on acetylcholine (ACh)-evoked CA secretory responses from the perfused rat adrenal glands. The CA secretion by a single injection of ACh (5.32 mM) in a volume of 0.05 ml was evoked at 15 min intervals for 90 min after loading with 150 μM of captopril as indicated at an arrow mark. Statistical difference was obtained by comparing the corresponding control (CONTROL) with captopril-treated group. Other legends are the same as in Fig. 2. **: P < 0.01.

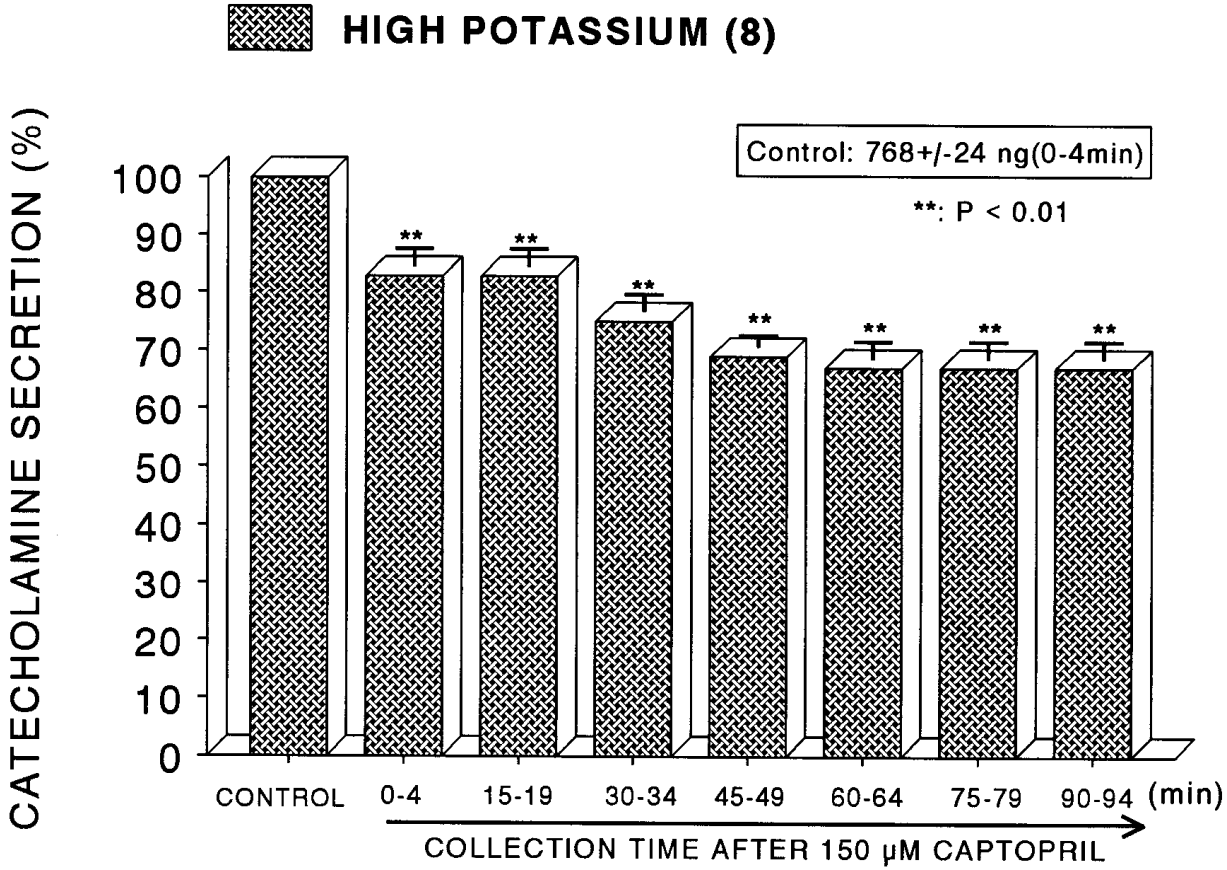


Fig. 11. Time-course effect of captopril on high potassium-evoked CA secretory responses from the perfused rat adrenal glands. The CA secretion by a single injection of K^+ (56 mM) in a volume of 0.1 ml was evoked at 15 min intervals for 90 min after loading with 150 μM of captopril. Statistical difference was obtained by comparing the corresponding control (CONTROL) with captopril-treated group. K^+-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 2. **: $P < 0.01$.

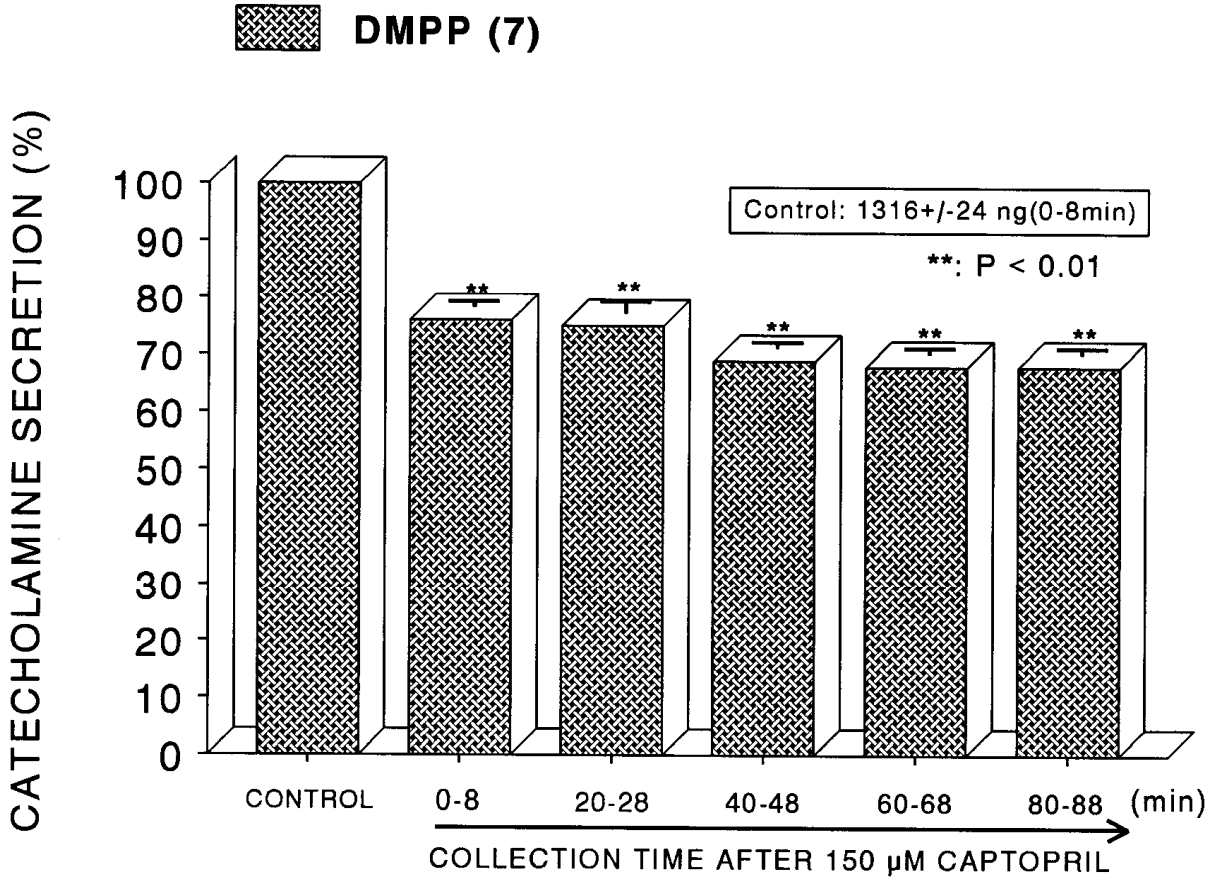


Fig. 12. Time-course effect of captopril on DMPP-evoked CA secretory responses from the perfused rat adrenal glands. The CA secretion by the perfusion of DMPP for 1 min was evoked at 20 min intervals for 90 in after loading with 150μM of captopril. Statistical difference was obtained by comparing the corresponding control with captopril-treated group. DMPP-induced perfusate was collected for 8 minutes. Other legends are the same as in Fig. 2. **: P < 0.01.

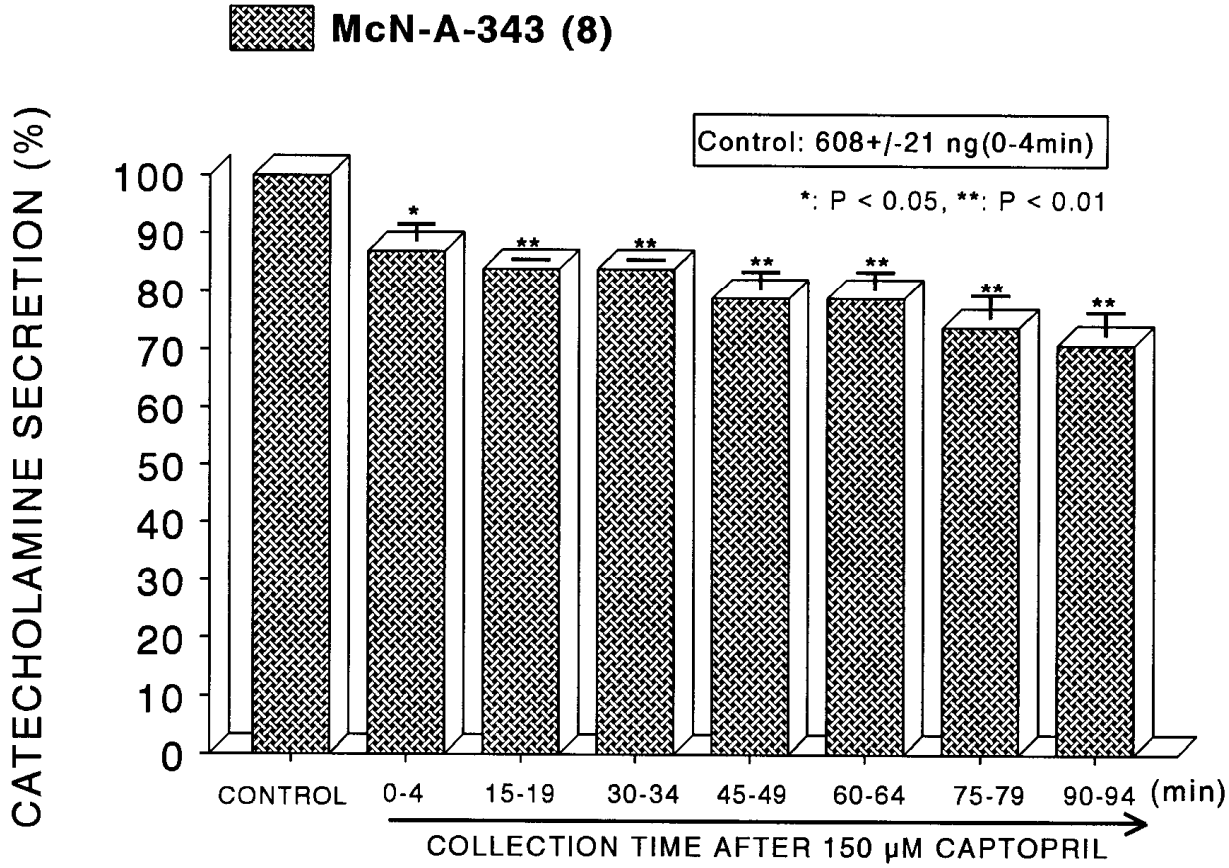


Fig. 13. Time-course effect of captopril onMcN-A-343-evoked CA secretory responses from the perfused rat adrenal glands. The CA secretion by the perfusion of McN-A-343 (100 μM) was evoked for 4 min at 15 min intervals for 90 min after loading with captopril (150 μM). Statistical difference was obtained by comparing the corresponding control with captopril-treated group. McN-A-343-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 2. *: P < 0.05, **: P < 0.01.

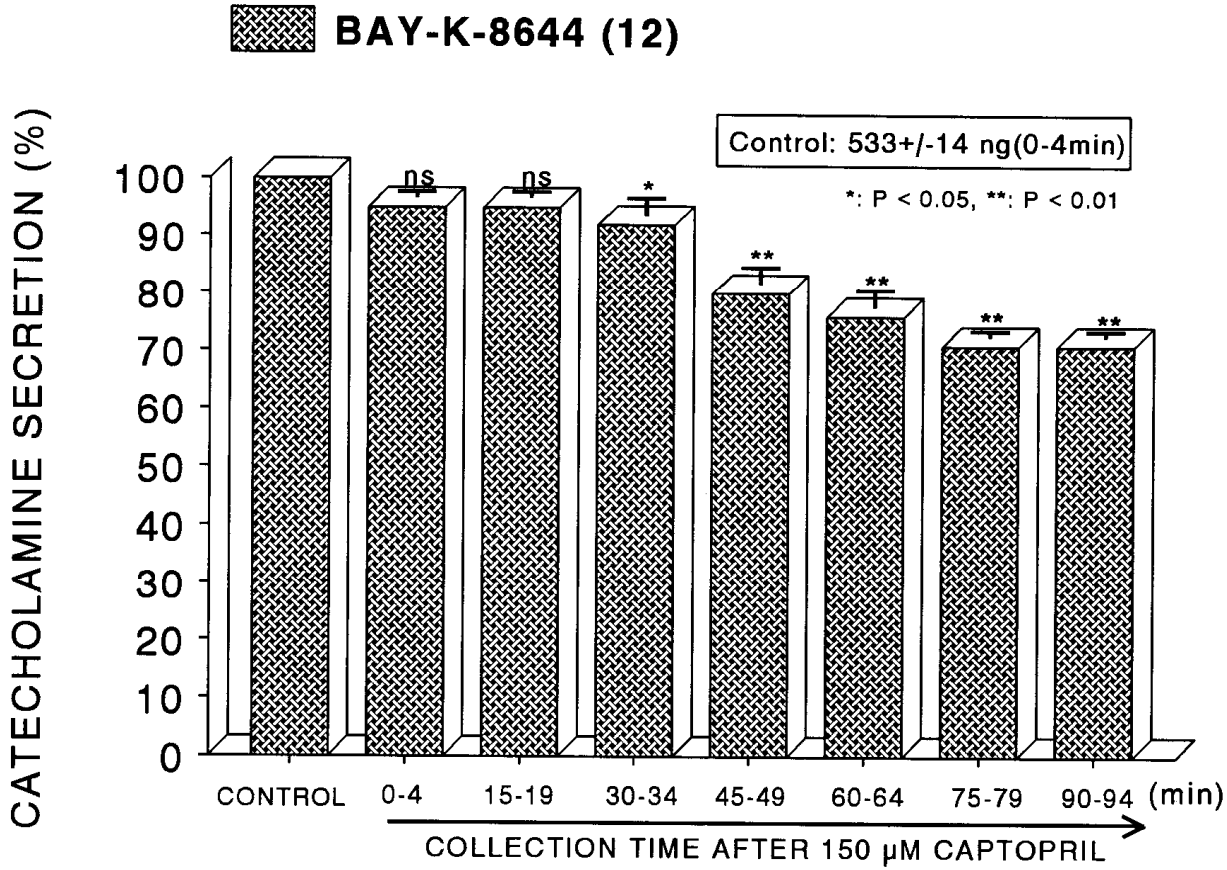


Fig. 14. Time-course effects of captopril on Bay-K-8644 evoked CA release from the perfused rat adrenal glands. Perfusion of Bay-K-8644 (10 μM) for 4 min was made at 15 min intervals for 90 min after loading with captopril (150 μM). Statistical difference was obtained by comparing the corresponding control with each period after treatment with captopril. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

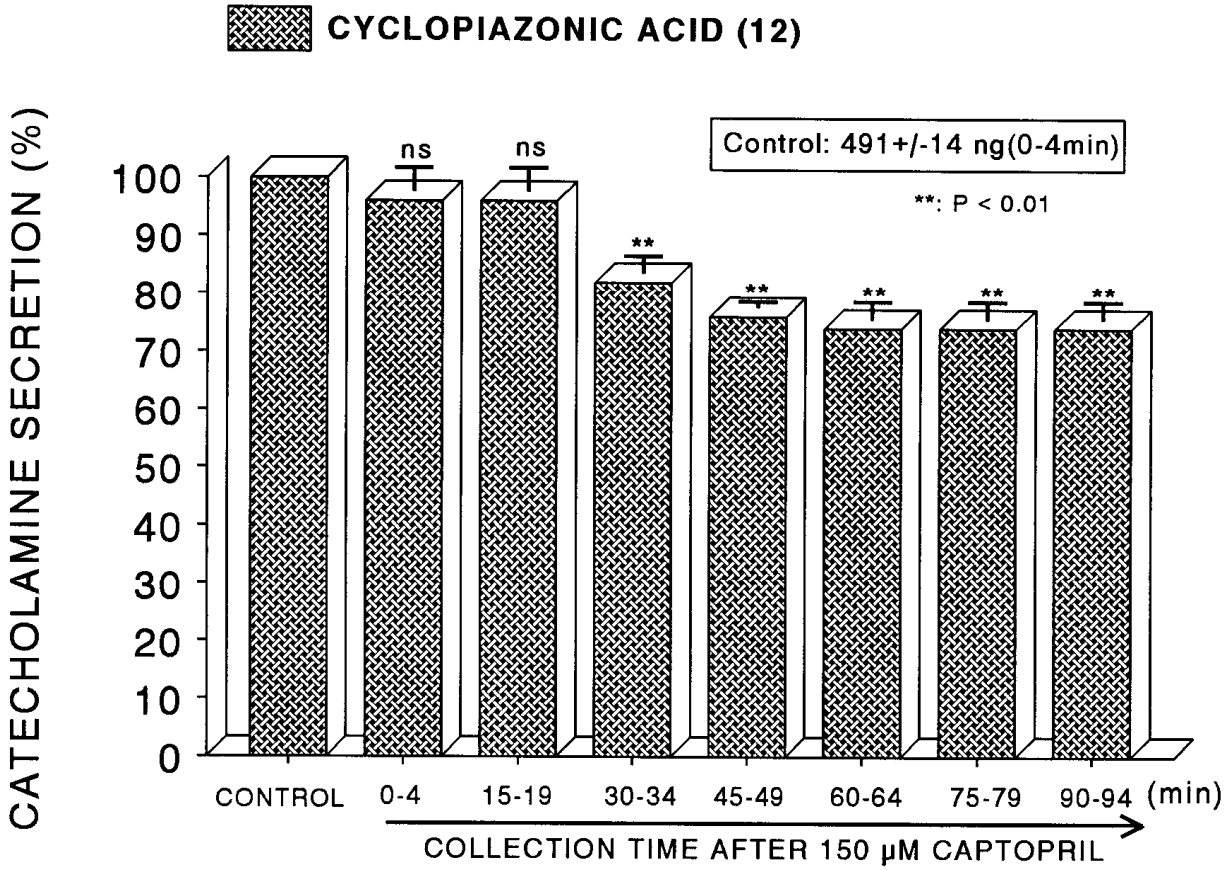


Fig. 15. Time-course effects of captopril on cyclopiazonic acid-evoked CA release from the perfused rat adrenal glands. Perfusion of cyclopiazonic acid (10 μM) for 4 min was made at 15 min intervals for 90 min after loading with captopril (150 μM). Statistical difference was obtained by comparing the corresponding control with each period after treatment with captopril. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns: Statistically not significant.

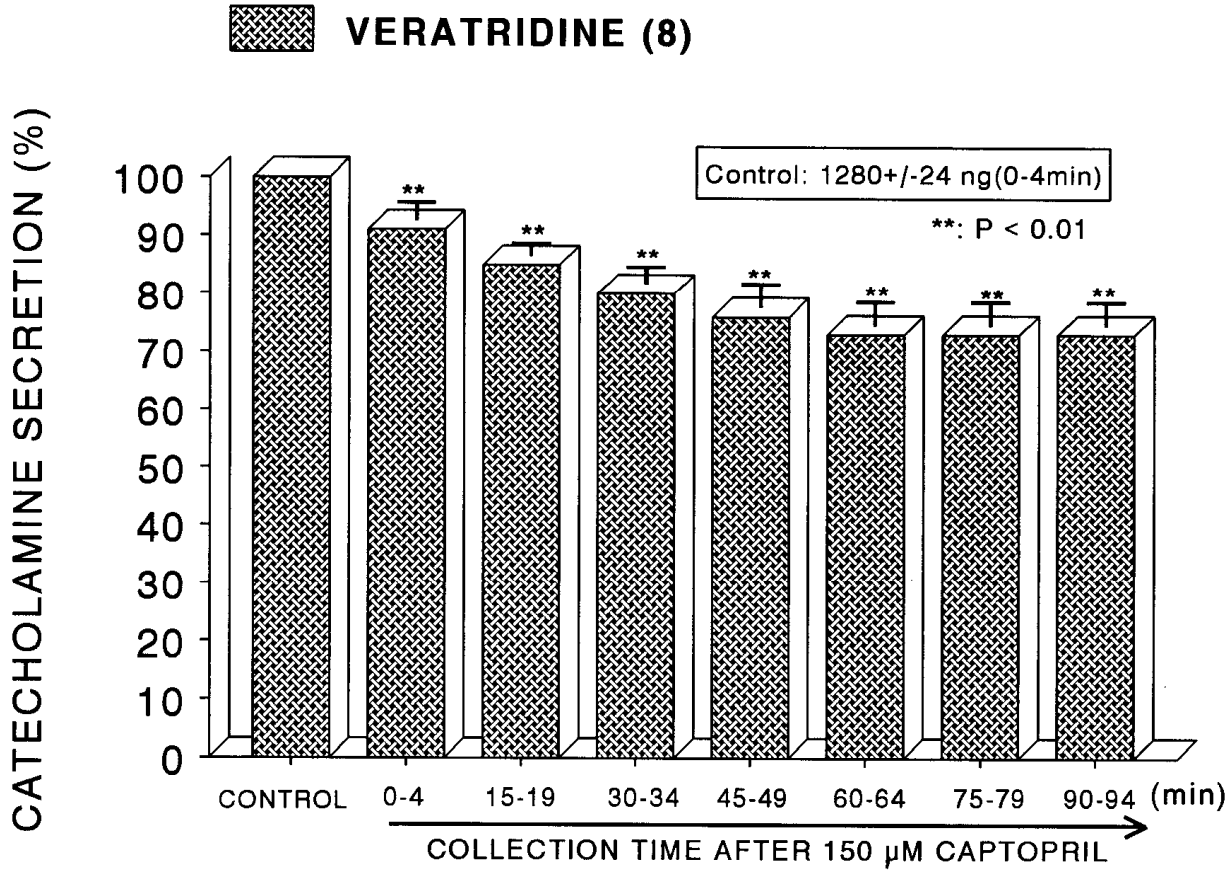


Fig. 16. Time-course effects of captopril on veratridine-evoked CA release from the perfused rat adrenal glands. Perfusion of veratridine (100 μM) for 4 min was made at 15 min intervals for 90 min after loading with captopril (150 μM). Other legends are the same as in Fig. 2. **: P < 0.01.

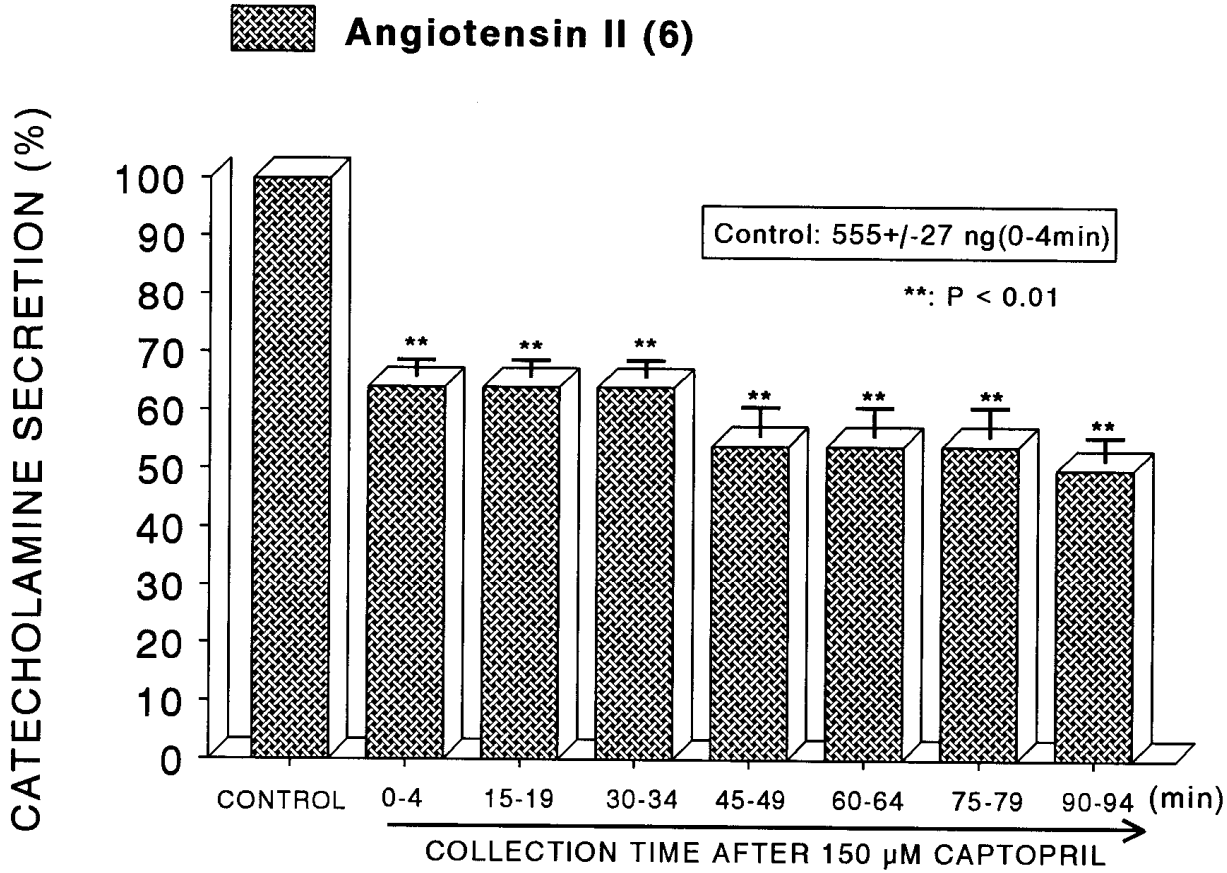


Fig. 17. Time-course effects of captopril on angoitensin II-evoked CA release from the perfused rat adrenal glands. Pefusion of angiotensin III (100 ηM) for 1 min was made at 15 min intervals for 90 min after loading with captopril (150 μM). Other legends are the same as in Fig. 2. **: P < 0.01.

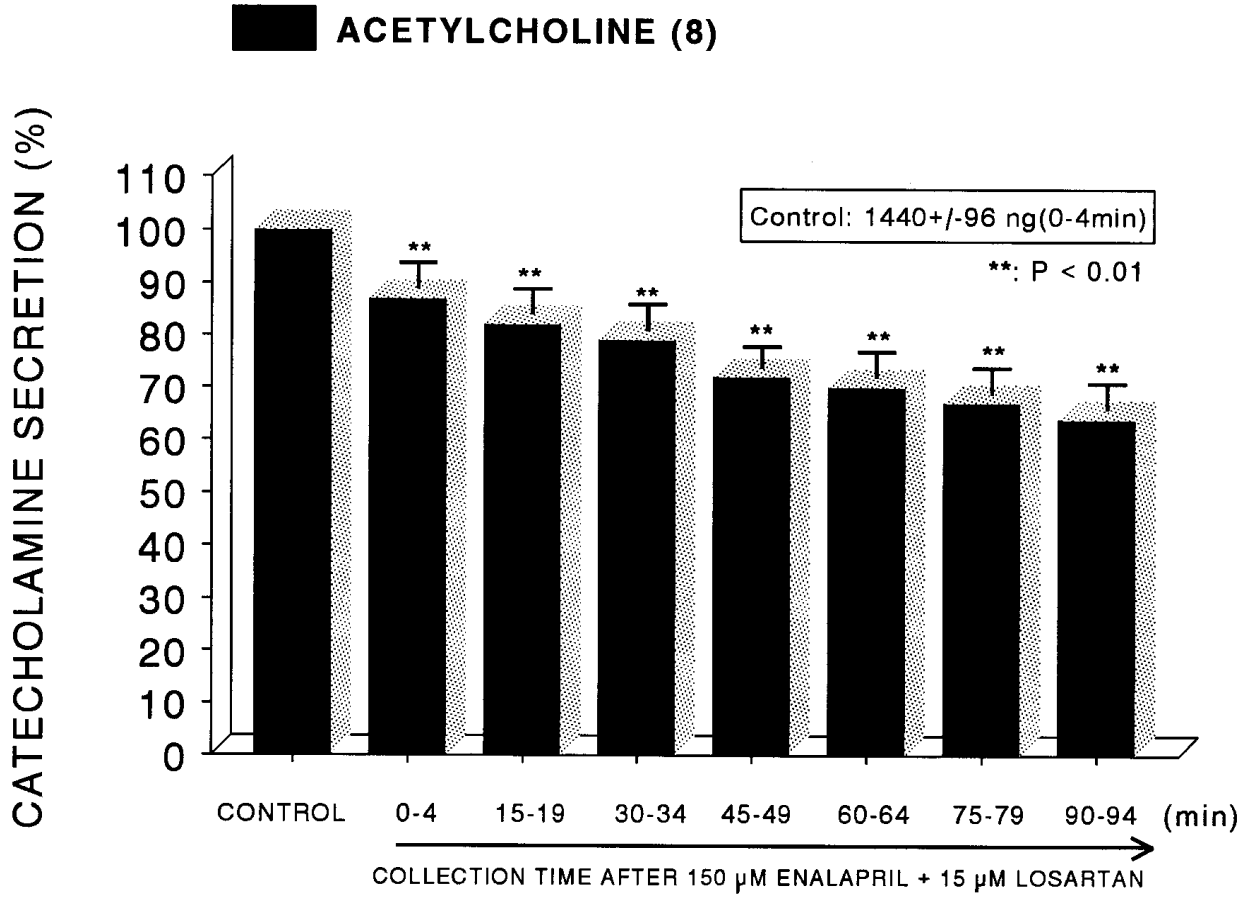


Fig. 18. Effects of enalapril plus losartan on the CA secretory responses evoked by acetylcholine from the isolated perfused rat adrenal glands. The CA secretion by a single injection of ACh (5.32 mM) in a volume of 0.05 ml was evoked at 15 min intervals for 90 min after simultaneous loading with 150 μM enalapril plus 15 μM losartan as indicated at an arrow mark. ACh-induced perfusate weas collected for 4 minutes. **: P<0.01.

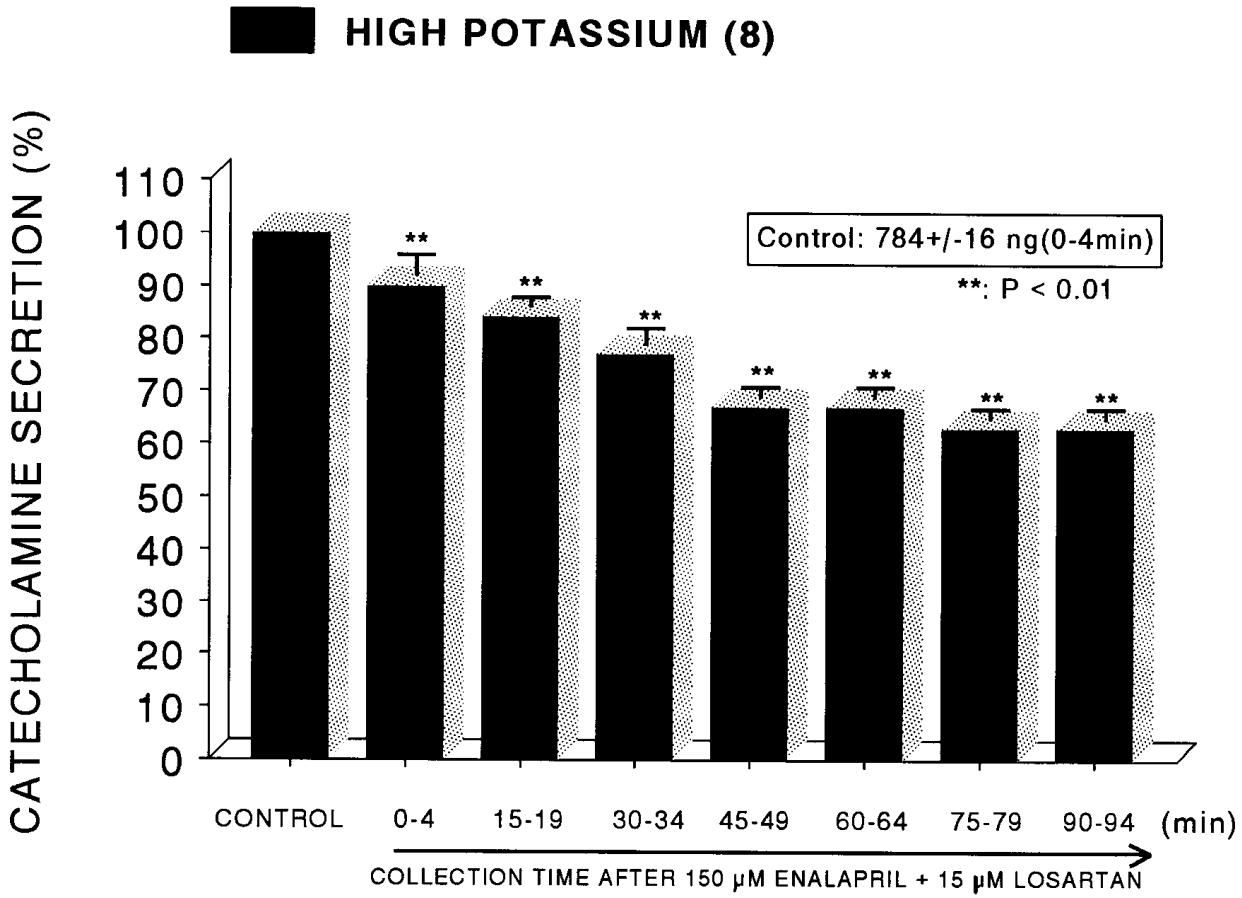


Fig. 19. Effect of enalapril plus losartan on the CA secretory responses evoked by high potassium from the isolated perfused rat adrenal glands. The CA secretion by a single injection of high potassium (56 mM) in a volume of 0.1 ml was evoked at 15 min intervals for 90 in after simultaneous loading with 150 μM enalapril plus 15 μM losartan as indicated at an arrow mark. High potassium-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 2. *: P< 0.05. **: P<0.01. ns: Statistically not significant.

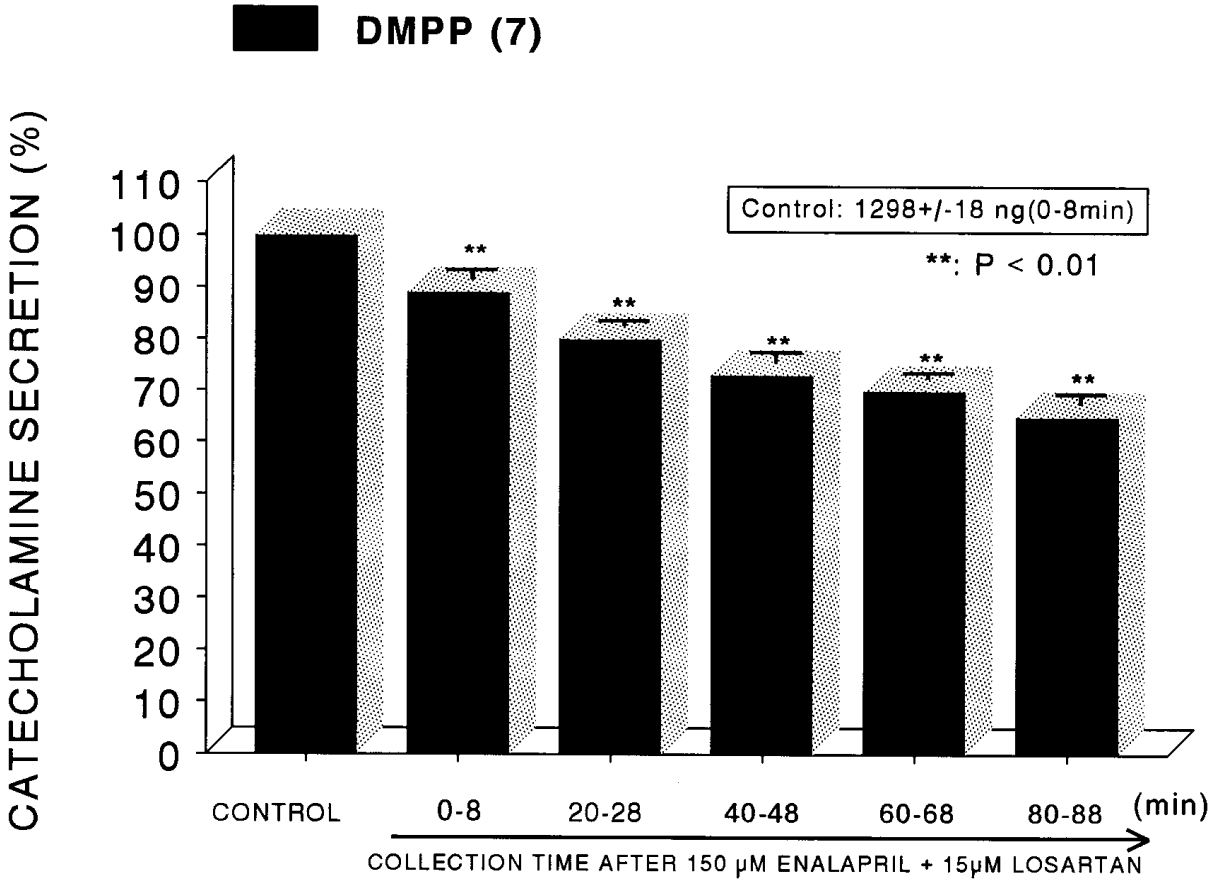


Fig. 20. Effect of enalapril plus losartan on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. The CA secretion by the infusion of DMPP (100 μM) for 1 min was evoked at 20 min intervals for 90 min after simultaneous loading with 150 μM enalapril plus 15 μM losartan. DMPP-induced perfusate was collected for 8 minutes. Other legends are the same as in Fig. 2. **: P<0.01.

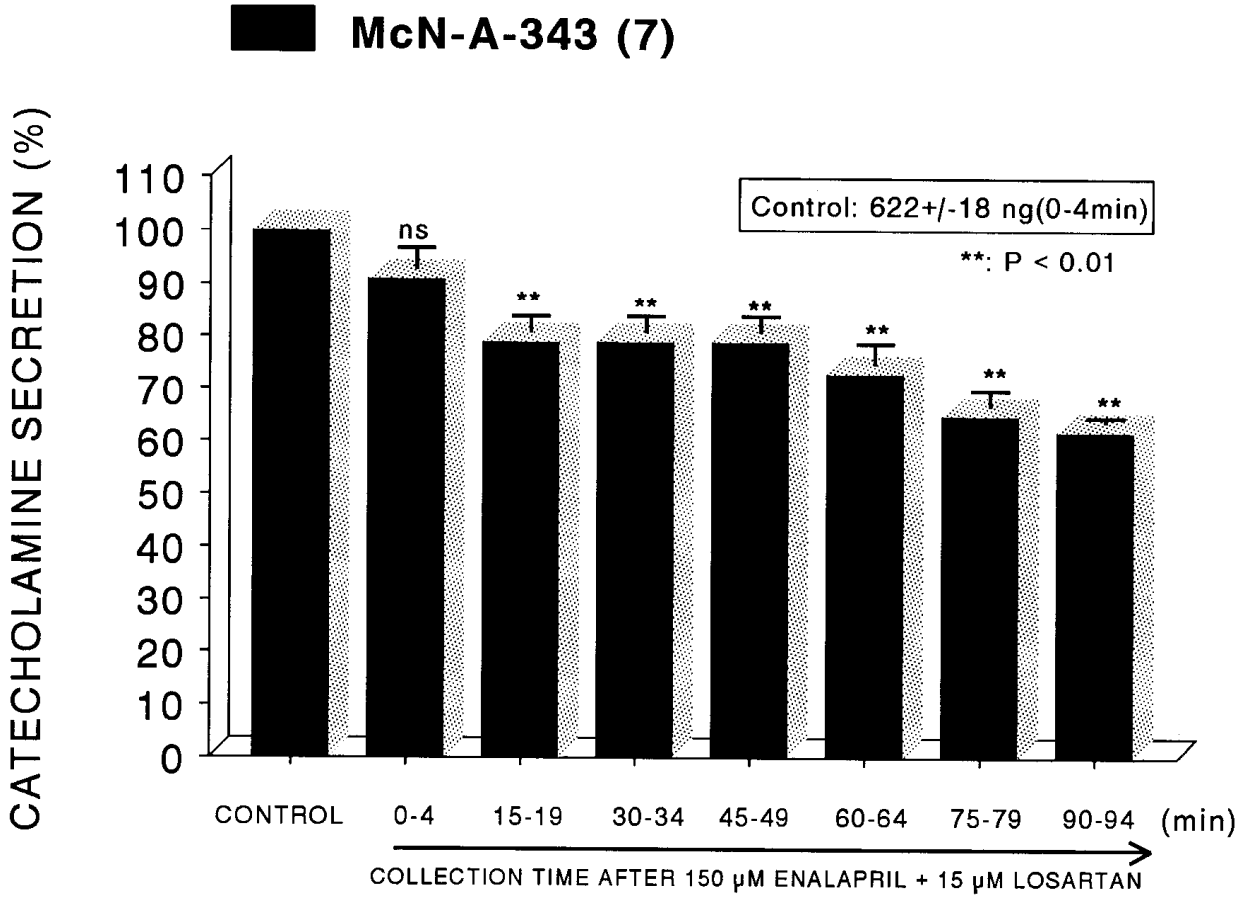


Fig. 21. Effect of enalapril plus losartan on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. The CA secretion by the infusion of McN-A-343 (100 μM) for 4 min was evoked at 15 min intervals for 90 min after simultaneous loading with 150 μM enalapril plus 15 μM losartan. McN-A-343-induced perfusate was collected for 4 minutes. Other legends are the same as in Fig. 2. **: P<0.01, ns: Statistically not significant.

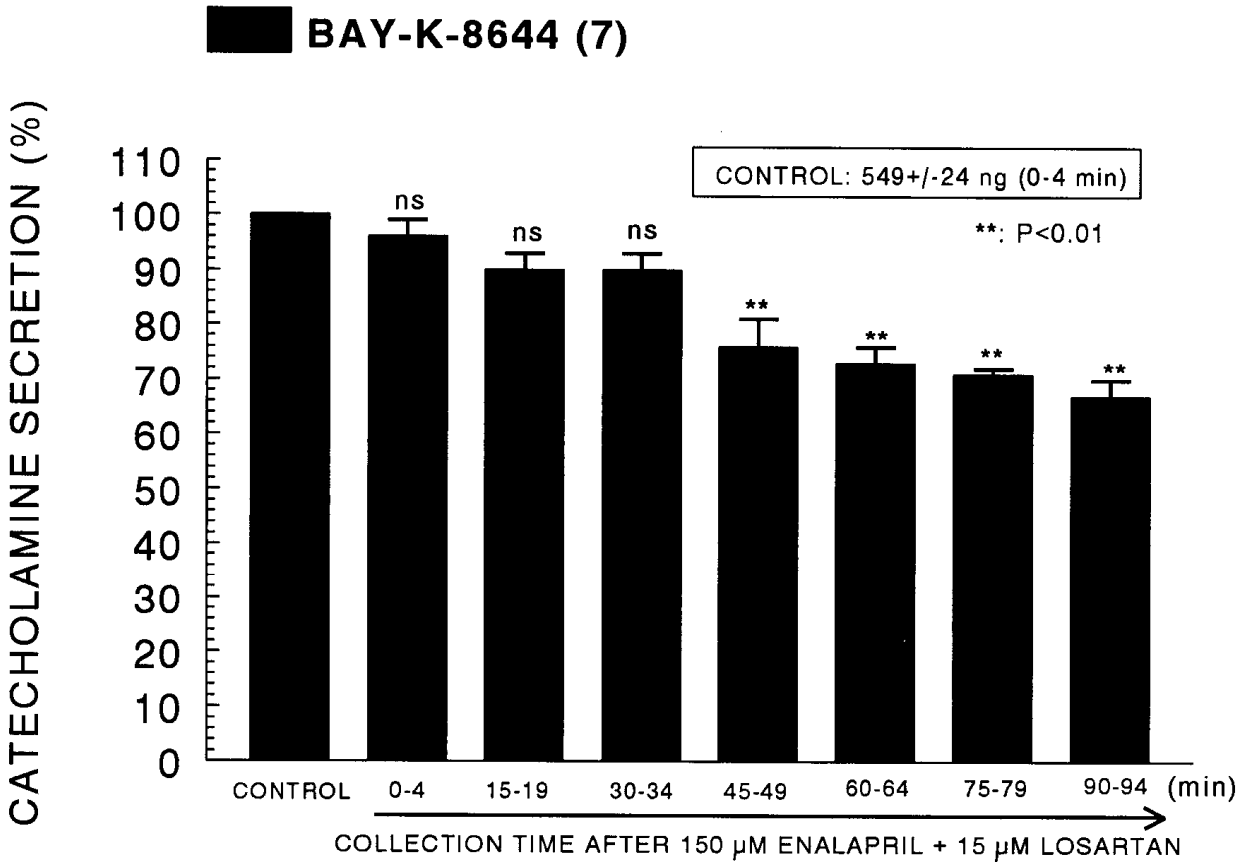


Fig. 22. Effect of enalapril plus losartan on catecholamine release evoked by Bay-K-8644 from the perfused rat adrenal glands. Perfusion of Bay-K-8644 (100 μM) for 4 min was made at 15 min intervals for 90 min after simultaneous loading with 150 μM enalapril plus 15 μM losartan. Other legends are the same as in Fig. 2. **: P<0.01, ns: Statistically not significant

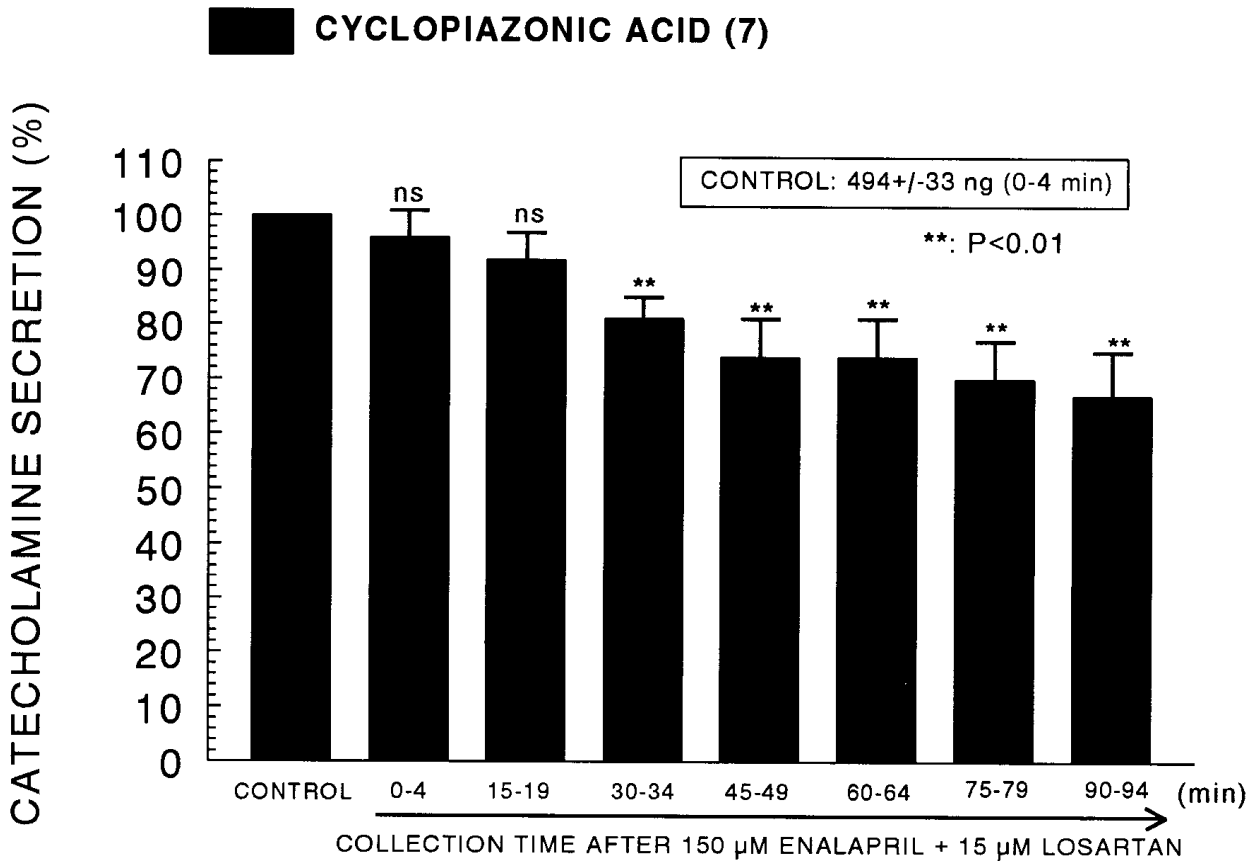


Fig. 23. Effect of enalapril plus losartan on catecholamine release evoked by cyclopiazonic acid from the perfused rat adrenal glands. Perfusion of cyclopiazonic acid (10 μM) for 4 min was made at 15 min intervals for 90 min after simultaneous loading with 150 μM enalapril plus 15 μM losartan. Other legends are the same as in Fig. 2. **: P<0.01. ns: Statistically not significant.

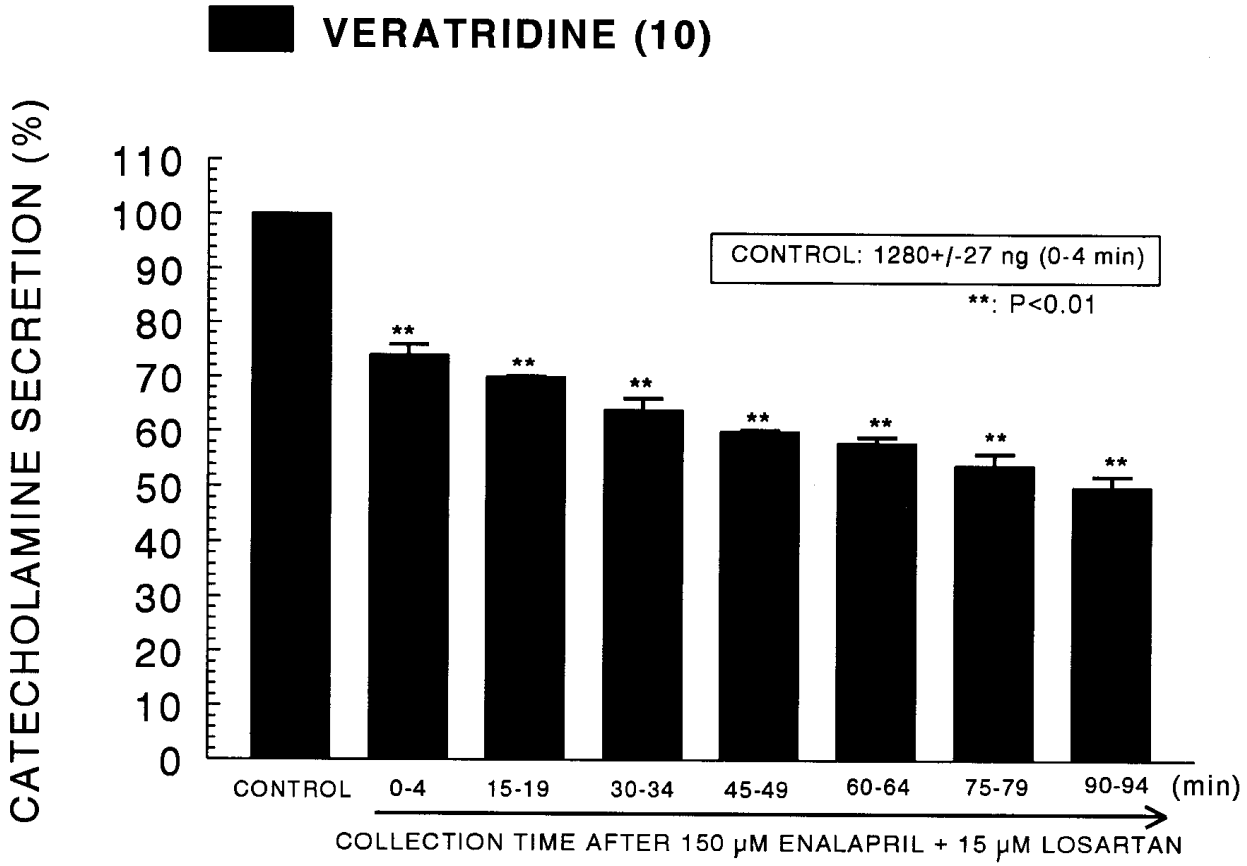


Fig. 24. Effect of enalapril plus losartan on catecholamine release evoked by veratridine from the perfused rat adrenal glands. Perfusion of veratridine (10^{-4} M) for 4 min was made at 15 min intervals for 90 min after simultaneous preloading with 150 μM enalapril plus 15 μM losartan. Other legends are the same as in Fig. 2. **: P<0.01.

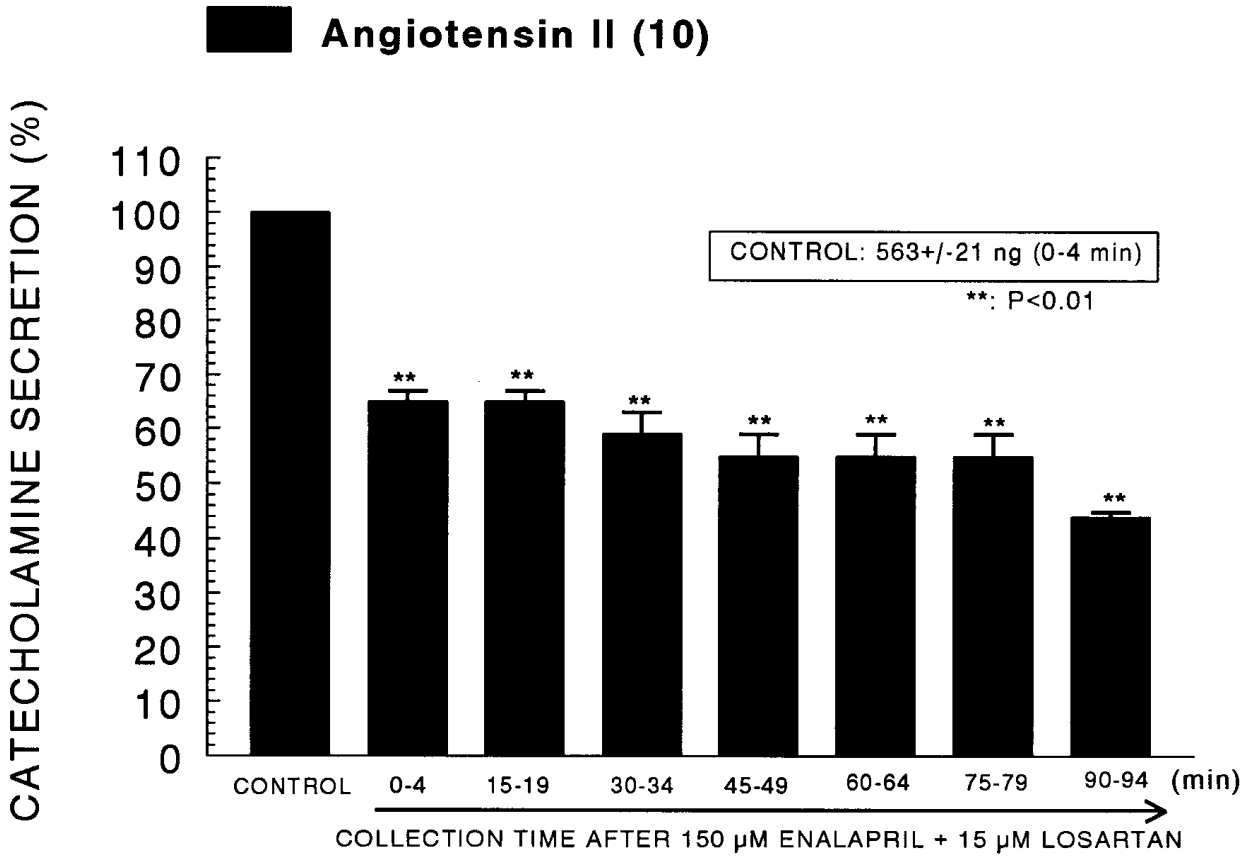
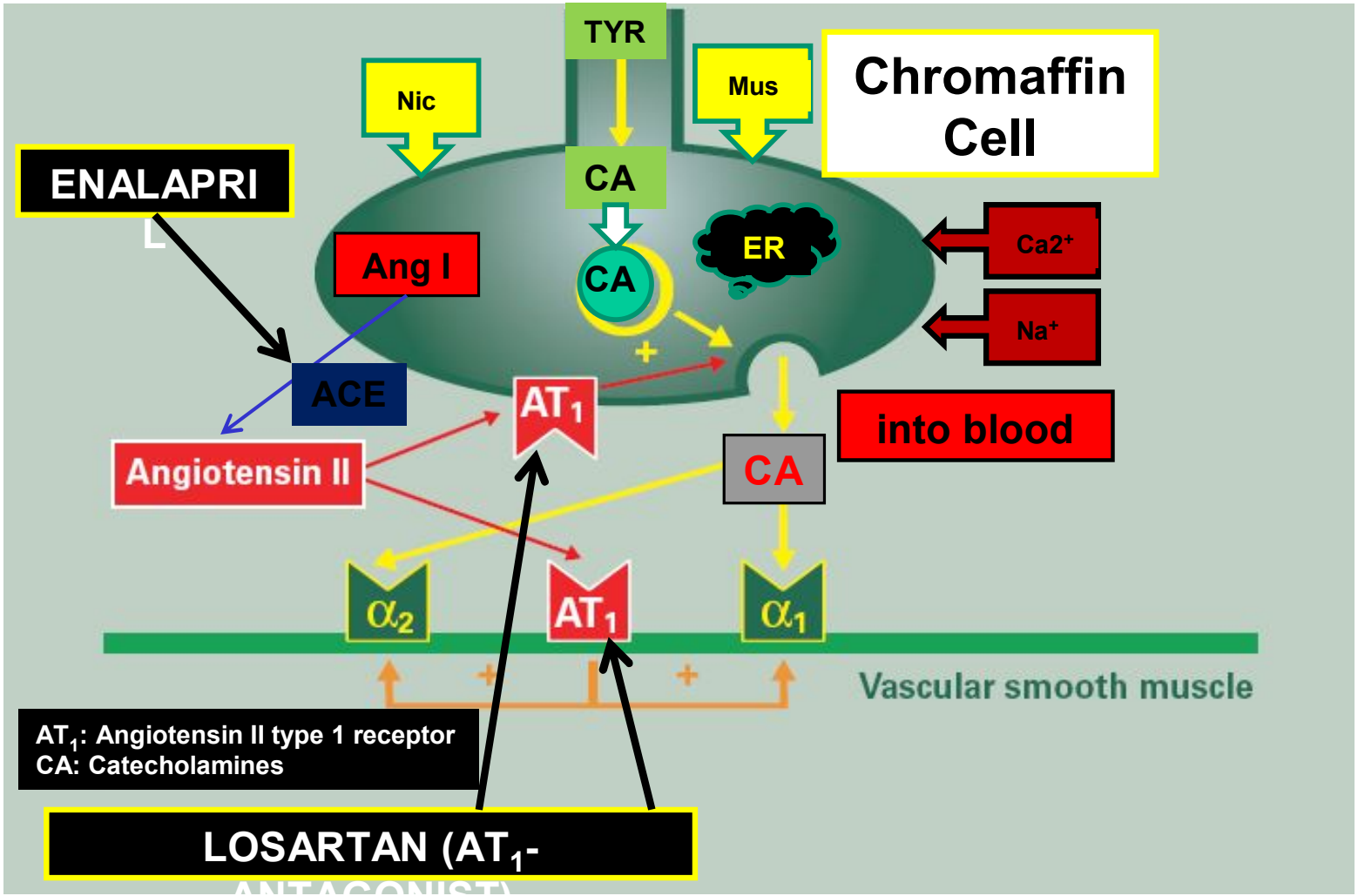


Fig. 25. Effect of enalapril plus losartan on catecholamine release evoked by angiotensin II from the rat adrenal enalapril plus losartan glands. Perfusion of angiotensin II (100 ηM) for 1 min was made at 15 min intervals for 90 min after simultaneous loading with 150 μM enalapril plus 15 μM losartan. Other legends are the same as in Fig. 2. **: P<0.01.

Fig. 26. Schematic diagram of possible action site of enalapril and losartan at the cholinergic nerve ending-chromaffin cell synapse in the rat adrenal gland



저작물 이용 허락서

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논문제목	한글 : 안지오텐신-전환효소억제제가 흰쥐 부신수질에서 니코틴 흥분작용에 의한 카테콜아민 분비에 미치는 영향 영어 : Influence of ACE Inhibitor on Nicotinic Stimulation-Evoked Catecholamine Secretion in the Perfused Rat Adrenal Medulla				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(0) 반대( )

2010년 2월

저작자: **서 유 석** (서명 또는 인)

조선대학교 총장 귀하