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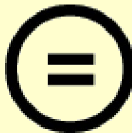

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Effects of AT_2-Receptor Antagonist on Catecholamine Release in the Isolated Perfused Rat Adrenal Medulla

조선대학교 대학원

의학과

Bhandary Bidur

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흰쥐 적출부신수질에서 AT_2 수용체 길항제가 카테콜아민 유리에 미치는 영향

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이 논문을 의학 석사학위신청 논문으로 제출함

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<국문 초록>

흰쥐 관류부신수질에서 AT_2수용체 길항제가 카테콜아민 유리에 미치는 영향

반다리 비두르

(지도교수: 임 동 윤)

조선대학교 대학원 의학과

이전의 일부 연구에서 Angiotensin II (AngII)의 카테콜아민(CA) 유리작용은 흰쥐 부신에서 얻은 부신수질조각(Belloni 등, 1998; Mazzocchi 등, 1998) 및 돼지 배양 부신수질세포 (Takekoshi 등, 2001)에서 AT_2 수용체 활성화를 통해서 나타나는 것으로 증명되었다. 이와는 대조적으로 AT_2 수용체 작동제인 CGP42112 는 돼지 배양 부신수질세포에서 CA 생합성을 억제하는 것으로 알려져 있으며 (Takekoshi 등, 2000), Martineau 등(1999)은 AT_2 수용체가 마취개의 부신수질로 AngII 수용체 작동제 투여에 의한 CA 분비를 나타내는데 역할을 한다고 하였다. 이와같이 부신에서 AT_2 차단제의 CA 분비에 대한 작용에 관해서 일부 논란이 있는 것 같다. 따라서 본 연구의 목적은 흰쥐 부신의 적출관류모델에서 선택성 AT_2 수용체 차단제인 PD123319 가 카테콜아민 유리에 영향을 미치는지를 검색하고자 본 연구를 시행하여 다음과 같은

결과를 얻었다. PD123319 (5-50 μM)을 부신정맥 내로 90 분간 관류 시 비교적 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 15 μM PD123319 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, 100nM)에 의한 CA 분비반응이 뚜렷이 억제되었다. 그러나 PD123319 및 CGP42112 자체는 기초 CA 분비량에 영향을 미치지 않았다. 이와 같은 연구결과를 종합하여 보면, 흰쥐 적출 관류 부신수질에서 PD123319 및 CGP42112 는 AngII 뿐만 아니라 콜린성(니코틴 및 무스카린 수용체) 흥분작용 및 직접 막탈분극에 의한 CA 분비작용을 유의하게 억제함을 제시하였다. 이들 두약물은 흰쥐 부신수질의 니코틴 수용체에서 길항작용을 나타내는 것으로 여겨진다. 또한 이러한 PD123319 및 CGP42112 의 CA 분비 억제작용은 흰쥐 적출 부신수질의 크롬친화세포내로 전압의존성 Na^{+} 및 Ca^{2+} 이온통로를 통한 세포내로 이들 이온의 유입을 차단하고 세포질내 칼슘저장고로부터 칼슘유리를 억제함으로서 나타나며, 이는 AT_2 수용체 차단작용을 통해 매개되는 것으로 사료된다. 이와 같은 연구결과로 보아, PD123319 및 CGP42112 는 흰쥐 적출 부신수질의 관류모델에서 작동제 작용이 없으며, AT_2수용체가 흰쥐 부신수질의 CA 분비에 관여하는 것으로 생각된다.

I. INTRODUCTION

Angiotensin II (AngII) is the principal effector hormone of the renin–angiotensin system, a system that plays a major role in the control of peripheral vascular resistance, blood pressure, and fluid and electrolyte homeostasis. AngII is known to exert its actions through at least two major subtypes of receptors, AngII type 1 (AT_1) and type 2 (AT_2) receptors, localized on the surface of plasma membrane of various tissues throughout the body (Timmermans et al., 1993; Mukoyama et al., 1993; Kambayashi et al., 1993; Unger et al. 1996; Ardaillou 1999). Ang II is a secretogogue for secretion of catecholamines (CA) that is believed to be mediated through IP_3 production by AT_1 (Wong et al., 1990; Dendorfer et al., 1998). Indeed, Ang II-induced CA release has been shown to be mediated by AT_1 in the rat adrenal medulla (Wong et al., 1990). 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 (Dendorfer et al., 1998). Moreover, it has been found that addition of IP_3 to permeabilized bovine chromaffin cells releases intracellular Ca^{2+} (Stoehr et al., 1986). Also, addition of Ca^{2+} to permeabilized bovine chromaffin cells was reported to cause CA secretion (Dunn and Holz, 1983). In the adrenal medulla, AngII increases the basal CA secretion through the activation of AT_1 receptors either in vitro (Balla et al. 1991; Timmermans et al. 1993; Hano et al. 1994) or in vivo (Martineau et al. 1995). However, a number of previous studies have indicated that the selective blockade of AT_1 receptors failed to abolish the increase in adrenal CA secretion

induced by AngII (Bunn and Marley 1989; Powis and O'Brien 1991; Wong et al. 1990; Martineau et al. 1995). These observations suggest the existence of additional mechanisms other than those mediated through AT_1 receptors. Generally, both AT_1 and AT_2 Ang II receptors are expressed in the adrenal medulla. In the rat, AT_2 receptors predominate, AT_1 receptors representing only 5–10% of the total number of Ang II receptors (Israel et al., 1995; Lu et al. 1995). Indeed, AT_2 is also abundantly expressed in the adrenal medulla of adult rats (Chiu et al., 1989; Whitebread et al., 1989). Previous studies have demonstrated that the CA-releasing effect of AngII is mediated primarily via the activation of AT_2 receptors in adrenal medullary fragments obtained from the rat adrenal gland (Belloni et al. 1998; Mazzocchi et al. 1998), and also in cultured porcine adrenal medullary chromaffin cells (Takekoshi et al., 2001). In contrast, it has been suggested that the AT_2 agonist, CGP 42112, inhibits CA biosynthesis through a decrease in cGMP production in cultured porcine adrenal medullary cells (Takekoshi et al., 2000). Martineau and his co-workers (1999) have shown that AT_2 receptors play a role in mediating CA secretion by the adrenal medulla of anesthetized dogs in response to AngII receptor agonist administration *in vivo*. PD 123319 (AT_2 antagonist) and CGP 42112 (AT_2 agonist) inhibited the increase in adrenal CA secretion induced by local administration of AngII without any agonist actions (Martineau et al., 1999). Nevertheless, such a functional implication of AT_2 receptors in the isolated adrenal medulla has yet to be confirmed in other species and. Therefore, the present study was designed to evaluate whether AT_2 receptors are functionally involved in the CA secretion in the perfused model of the isolated rat adrenal medulla. The specific purpose of the present study was to analyze modulations of adrenal CA secretion from the

perfused rat adrenal gland in response to locally administered several secretagogues including AngII in the presence of either PD 123319 or CGP 42112, both of which are highly specific and selective ligands to angiotensin AT_2 receptor in various tissues (Nahmias and Stroberg 1995).

II. MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 200 to 300 grams, were anesthetized with CGP42112 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 $\pm 1^{o}$C (Fig. 2).

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 0.33 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 Ang II (10^{-7} M) and DMPP (10^{-4} M) for 1~2 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 angiotensin II, 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 PD123319 on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing PD123319 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 PD123319, 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: PD123319, cyclopiazonic acid, 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), veratridine hydrochloride, angiotensin II acetate salt (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

Influence of PD123319 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 21 ± 2 ng for 2 min (n=9). Since Martineau and hid colleagues (1999) suggested that AT_2 receptors play a role in mediating catecholamine secretion from the adrenal gland of anesthetized dogs by the adrenal medulla in response to Ang II receptor agonist administration, it was attempted initially to examine the effects of PD123319 itself on the CA secretion from the perfused model of the rat adrenal glands. However, in the present study, PD123319 (10^{-5} ~ 10^{-4} 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 PD123319 on the CA secretory responses evoked by ang II, cholinergic receptor stimulation, and membrane depolarization. Secretagogues were given at 15~20 min-intervals. PD123319 was present for 90 minutes after the establishment of the control release.

When ACh (5.32 x 10^{-3} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 1458±82 ng for 4 min. However, in the presence of PD123319 in the range of 5 ~ 50 nM for 90 min, ACh-stimulated CA secretion was inhibited by PD123319 in concentration- and time-dependent fashion. As shown in Fig. 2, in the presence of PD123319, ACh-evoked CA releasing responses were inhibited by ~71% of the

corresponding control release. Also, the direct membrane-depolarizing agent, high potassium markedly evoked the CA secretion (859±23 ng for 0-4 min). However, following the pretreatment with PD123319 (5 ~ 50 nM), high K^+ (5.6 x 10^{-2} M)-stimulated CA secretion was significantly inhibited by ~69% of the control at last period (90-94 min) as shown in Fig. 3. DMPP (10^{-4} M), which is a selective nicotinic (N_N) receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (1358±32 ng for 0-8 min). However, as shown in Fig. 4, DMPP-evoked CA secretion after pretreatment with PD123319 was also maximally reduced to 71% of the control release at last period. McN-A-343 (10^{-4} M), 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 (569±26 ng for 0-4 min). However, in the presence of PD123319, McN-A-343-evoked CA secretion was markedly depressed to ~43% of the corresponding control secretion (Fig. 5).

Influence of PD123319 on the 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 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 PD123319 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 PD123319 (15 nM) was greatly blocked to 63% of the control at 75-94 min period as compared to the

corresponding control release (525±13 ng for 0-4 min) from 10 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 PD123319 on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 7. In the presence of PD123319 (15 μM) from 10 adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also inhibited to 70% of the control response (512±10 ng for 0-4 min) at 45-94 min period.

The voltage-dependent Na^{+} channels consist of the principal α-subunit, which is associated with a noncovalently attached β_1-subunit, 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 PD123319 on voltage-dependent Na^{+} channels, the effect of PD123319 on veratridine-evoked CA secretion was examined here. As shown in Fig. 8, veratridine (10^{-4} M) greatly produced the CA secretion (1395±30 ng for 0-4 min). However, in the presence of PD123319 (15 μM), veratridine-evoked CA secretion was greatly inhibited to 66% of the corresponding control release.

Since it has been demonstrated that AT_2 stimulation induces CA secretion in cultured porcine chromaffin cells (Takekoshi et al., 2001), but Hano and his colleagues (1994) have suggested that AngII increase epinephrine release from

the adrenal medulla via the AT_1 receptors, it was likely interesting to examine the effect of AngII on the CA rease. AngII (10^{-7} M) significantly evoked the CA secretory response (706±24 ng for 0-4 min) whereas, in the presence of PD123319 (15 μM), AngII (100 nM)-evoked CA secretion was greatly inhibited to 59% of the corresponding control release (Fig. 9).

Influence of CGP42112 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, PD123319 inhibited the CA secretory response evoked by cholinergic stimulation in the perfused rat adrenal gland. It has also been demonstrated that AT_2 receptpr agonist (CGP 42112) reduces both TH-enzyme activity and TH-synthesis in cultured porcine adrenal medullary cells and that these inhibitory effects could be mediated by decrease of cGMP production. Therefore, in order to compare the effect of CGP42112 with that of PD123319, it was likely of interest to examine effect of CGP42112 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, CGP42112 (15 nM) itself did not affect basal CA output from perfused rat adrenal glands (data not shown). In subsequent experiments, CGP42112 (15 nM) 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 CGP42112 (15 nM) was 1320±43 ng (0-4 min) from 16 rat adrenal glands. However, in the presence of CGP42112 (15 nM) for 90 min, it was significantly attenuated to 75% of the control release

(Fig. 10). High K^+ (56 mM)-evoked CA release under the presence of CGP42112 (15 nM) was also reduced to 82% of the corresponding control secretion (882±45 ng, 0-4 min) from 9 glands, as shown in Fig. 11. In 12 rat adrenal glands, DMPP (10^{-4} M) perfused into the adrenal gland produced great CA secretion (1344±19 ng, 0-8 min) prior to loading with CGP42112. Following perfusion with CGP42112 (15 nM) it was diminished to 73% of the corresponding control release (Fig. 12). Moreover, in the presence of CGP42112 (15 nM), McN-A-343-evoked CA secretory responses was also time-dependently inhibited by 84% of the control secretion (640±10 ng, 0-4 min) from 10 glands, as shown in Fig. 13.

Influence of CGP42112 on the CA secretion evoked by Bay-K-8644, cyclopiazonic acid, veratridine and AngII from the perfused rat adrenal glands

In the presence of CGP42112 (15 nM), the secretory responses evoked by Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) given into the adrenal gland for 4 min were greatly depressed to 75% and 71% of their the corresponding control responses (512±48 ng for 4 min and 544±21 ng for 4 min), respectively (Fig. 14 and 15). The CA secretion evoked by veratridine (10^{-4} M), an activator of Na^+ channels, was greatly elevated to 1371±37 ng for 0-4 min before loading of CGP42112. However, in the presence of CGP42112, it was inhibited to 73% of the corresponding control secretion, as shown in Fig. 16. AngII (10^{-7} M) also markedly increased the CA secretory response (691±21 ng for 0-4 min) whereas, in the presence of CGP42112 (15 nM), AngII (100 nM)-evoked CA secretion was greatly inhibited to 70% of the corresponding control release (Fig. 17).

IV. DISCUSSION

These results obtained from the present study suggest that PD123319 can inhibit the CA secretion evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization from the rat adrenal medulla. This inhibitory effect of PD123319 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 Ang II AT1 receptors located on the presynatic membrane of the rat adrenomedullary chromaffin cells, which are relevant to adrenal nicotinic receptor blockade.

The present study has demonstrated that perfusion of both CGP 42112 and PD123319 into the adrenal gland, in time- and dose-dependent fashion, resulted in a significant decrease in the CA secretory responses evoked by cholinergic stimulation as well as by direct membrane-depolarization while CGP 42112 or PD123319 itself did not affect the basal CA secretion. The results of the present study also indicate that AngII-evoked CA release was reduced significantly in a time-dependent manner by a selective antagonist, PD123319, to angiotensin AT_2 receptors in the perfused rat adrenal gland. In support of this idea, it has been well established that PD123319 expresses highly selective and specific affinity to AT_2 receptors in various tissues, including the adrenal medulla (Bumpus et al. 1991; Timmermans et al. 1993). Furthermore, PD123319 is widely accepted to be a selective nonpeptide AT_2 receptor antagonist (de Gasparo et al. 1998). For example, in the rat portal vein, which contains both AT_1 and AT_2 receptors, AngII-induced contractions were partially inhibited by selective AT_2 receptor

blockade with PD123319 (Pelet et al. 1995). In the adrenal gland, AT_2 receptors have been implicated in cortisol secretion in response to AngII in bovine adrenal fasciculata cells in vitro as PD 123319 significantly diminished the secretion of cortisol induced by AngII (Defaye et al. 1995). Therefore, it seems that the inhibitory effect of PD 123319 on the CA release evoked by ACh, high K+, DMPP, McN-A-343, and AngII is due to AT_2 receptor blockade. This present observation is compatible with previous work demonstrating that PD123319 inhibits AngII-evopked CA release from rat adrenal medullary fragments (Belloni et al. 1998; Mazzocchi et al. 1998) as well as from the adrenal gland of the anesthetized dogs (Martneau et al., 1999). These in vitro and in vivo observations are consistent with this hypothesis that the CA secretagogue effect evoked by AngII as well as cholinergic stimulation and direct membrane-depolarization involves, at least in part, activation of AT_2 receptors localized on the rat adrenal medullary chromaffin cells. It has also been known that CGP42112 affects AT_2 receptors in tissues with a highly heterogeneous distribution of receptor subtypes by virtue of its high affinity, high selectivity, low nonspecific binding, and high stability (Whitebread et al. 1991; Heemskerk and Saavedra 1995). CGP42112 is considered to be a selective AT_2 agonist (de Gasparo et al. 1998). However, unlike PD 123319, the pharmacological activity of CGP 42112 is likely to vary depending on tissues and species studied. For example, CGP 42112 has been claimed to act as a full AT_2 agonist in intact PC12W cells (Brechler et al. 1993) and in the rat adrenal medullary fragments in vitro (Belloni et al. 1998). However, in the present work, the findings that CGP42112 significantly inhibited the CA release evoked by ACh, high K+, DMPP, McN-A-343, and AngII are not accordance with the results acting as a full AT_2 agonist. By contrast, CGP 42112

was found to behave as a selective AT_2 antagonist in cultured rat cardiac myocytes (Lokuta et al. 1994) as well as in the duck adrenal gland and subfornical organ in vitro (Schäfer et al. 1996). In the present study, CGP 42112, by itself, did not affect basal CA secretion at a dose used in this experiment. It has been previously shown that AngII markedly increased the basal CA secretion in a dose-dependent manner within the dose range similar to that used for CGP 42112 (Martineau et al. 1995). The increase in adrenal CA output in response to AngII was inhibited by ~80% following the largest dose of PD 123319, and CGP42112 significantly attenuated the CA response to AngII by ~70% (Martineau et al., 1999). Furthermore, both PD 123319 and CGP 42112 inhibited the increase in adrenal CA secretion induced by local administration of AngII (Martineau et al., 1999). Recently, in isolated rat hearts, CGP-42112A has been shown to suppresse nerve stimulation-induced NE overflow in the same way as the combination of Ang II and losartan, and this suppression was abolished by PD-123319 (Sasaoka et al., 2008). Moreover, it has been shown that the binding potency of CGP42112 at AT_2 receptors is significantly greater than that of AngII (Heemskerk and Saavedra 1995). Therefore, the absence of any positive effect of CGP 42112 on the basal CA secretion is compatible with the view that CGP42112 is devoid of any agonist activity in the canine adrenal medulla in vivo (Martineau et al., 1999). However, the absence of any agonist activity on AT_2 receptors is sharply contrasted with the recent observation in rat adrenal medullary fragments in which basal CA release increased significantly in response to CGP 42112 in a concentration- dependent manner within the range from 0.001 mM to 1.0 mM (Belloni et al. 1998). The reason for this discrepancy is at present unclear. It may be accounted for by the different experimental

conditions (in vivo versus in vitro [perfused model]) or the different animal species (dog versus rat) employed in those studies. Indeed, chromaffin cells in adrenal medullary fragments in vitro may differ from those in adrenal medulla in vivo in their reactivity in response to AngII. For example, chromaffin cells in vitro had to be incubated with AngII for 90 min to obtain an appropriate CA response (Belloni et al. 1998), while adrenal medulla tissues studied in vivo are affected when exposed to AngII for only 1 min at an infusion rate of 0.5 mL/min (Martineau et al. 1995, 1996) and also the perfused rat adrenal gland increased the CA release when exposed to AngII for only 1 min at an perfusion rate of 0.31 mL/min (Noh et al., 2009). Furthermore, the distinctive reactivity of such tissues may differ among species. The present experiments demonstrated that adrenal CA release evoked by AngII as well as cholinergic stimulation and direct membrane depolarization is significantly attenuated in the presence of CGP42112. Takekoshi and his co-workers (2001) have shown that CGP 42112 (AT_2 agonist) reduces both TH-enzyme activity and TH-synthesis biosynthesis in cultured porcine adrenal medullary cells and that these inhibitory effects could be mediated by decrease of cGMP production. Because CGP42112 is highly selective to AT_2 receptors with low nonspecific binding (Whitebread et al. 1991; Heemskerk and Saavedra 1995), it is plausible that the CGP42112-induced inhibition of the CA release is primarily attributable to specific blockade of AT_2 receptors in the adrenal medulla. Based on the results obtained when studying the nonpeptidic AT_2 antagonist PD 123319, the present data support the hypothesis that AT_2 receptors are functionally involved in local regulation of CA secretion in the perfused rat adrenal medulla.

In contrast,, it has been reported that, in cultured porcine chromaffin cells, AT_2 stimulation induces CA secretion by mobilizing Ca^2 through voltage-dependent

Ca^{2+} channels without affecting intracellular pools and that these effects could be mediated by a decrease in cGMP production (Takekoshi et al., 2001). Worck and his colleagues (1998) have also speculated that angiotensin II through binding to both receptor subtypes (both AT_1 and AT_2) 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. In light of these results, the present findings seem to be disagreement with those results that adrenal CA secretion is mediated through AT_2 receptors. On the other hand, Armando and his colleagues (2004) have demonstrated that both adrenomedullary AT_1 and AT_2 receptor types maintain and promote the adrenomedullary CA synthesis and the transcriptional regulation of TH in rats. Instead of opposing effects, however, these results indicate a complex synergistic regulation between the AT_1 and AT_2 receptor types.

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 determine if the inhibition of DMPP-stimulated secretion by AT_2 antagonist was due to an effect on the activity of the nicotinic receptor, the effect of PD123319, on DMPP-stimulated CA secretion was examined. As shown in Fig. 4 and 12, treatment with PD123319 or CGP42112 greatly inhibited DMPP-evoked CA secretion, reducing by 71% and 73% of their control release, respectively. It is likely plausible that PD123319 or CGP42112 can activate a signal transduction pathway that is altering the activity of both nicotinic receptors and voltage-sensitive Na^+ channels.

In the present study, both PD123319 and CGP42112 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. The observation that AT_1-selective antagonist inhibited the CA secretion evoked by Bay-K-8644 was surprising, as Takekoshi et al. (2001) have reported that removal of external Ca^2 significantly suppressed either AngII plus CV-11974 (AT_1 antagonist, 100 nM; which simulates specific AT_2 stimulation) or CGP 42112 (AT_2 agonist)-induced CA secretion in cultured porcine adrenomedullary chromaffin cells. It is unclear how the blockade of AT_2 receptors results in the inhibition of secretion seen in these cells. The simplest interpretation is that the decrease in Ca^{2+} uptake by PD123319 or CGP42112 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 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 CA secretion. Consequently, although the decrease in Ca^{2+} uptake (influx) into the adrenal chromaffin cells may explain the decrease by PD123319- or CGP42112 in CA secretion, it is still unclear whether this is only or even most important factor contributing to the inhibition of CA secretion by the AT_2 antagonist. 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 PD123319 or CGP42112-mediated inhibition of CA secretion.

In the present study, 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 & Riley, 1989; Seidler et al., 1989). Therefore, it is felt that the inhibitory effect of PD123319 or CGP42112 on 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 blockade of AT_2 receptors causes 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, PD123319 or CGP42112 time- and concentration-dependently produced the inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests new other concept that PD123319 or CGP42112 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 reticulum, resulting in increase in the subsequent Ca^{2+} release from those storage sites and thereby increase of 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 losartan 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 AT_2 receptor blockade by losartan. Based on these previous results, this finding of the present work suggests that AT_2 receptor blockade-induced inhibition may be involved in regulating CA secretion evoked by muscarinic M_1-receptor stimulation in the rat adrenal medullary chromaffin cells.

Taken together, as shown in Fig. 18, these experimental results suggest that PD123319 or CGP42112 inhibits the CA secretion evoked by AngII as well as cholinergic stimulation (both nicotininc and muscarinic receptors) and direct membrane depolarization from the rat adrenal medulla. It seems that both PD123319 and CGP42112 have antagonist activity at nicotinic receptors of the perfused rat adrenal medulla. It is also thought that this inhibitory effect of both PD123319 and CGP42112 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 AT_2 receptor blockade. Based on these present data, it is suggested that both PD123319 and CGP42112 have no agonist activity in the perfused model of isolated rat adrenal medulla, and that AT_2 receptors may be involved in the rat adrenomedullary CA secretion.

V. SUMMARY

Previously some studies have demonstrated that the CA-releasing effect of AngII is mediated primarily via the activation of AT_2 receptors in adrenal medullary fragments obtained from the rat adrenal gland (Belloni et al. 1998; Mazzocchi et al. 1998), and also in cultured porcine adrenal medullary chromaffin cells (Takekoshi et al., 2001). In contrast, an AT_2 agonist, CGP 42112, has been shown to inhibit CA biosynthesis through a decrease in cGMP production in cultured porcine adrenal medullary cells (Takekoshi et al., 2000). Martineau and his co-workers (1999) have shown that AT_2 receptors play a role in mediating CA secretion by the adrenal medulla of anesthetized dogs in response to AngII receptor agonist administration *in vivo*. Thus, there seems to be some controversy about the effect of AT_2 receptor blockade on the CA secretion from the adrenal gland. The aim of this study therefore was to determine whether PD123319 could influence the CA release from the isolated perfused model of the rat adrenal medulla. PD123319 (5~50 nM) perfused into an adrenal vein for 90 min produced dose- and time-dependent inhibition in 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 PD123319 (15 nM) for 90 min, 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 Na^+ channels), and angiotensin II (Ang II, 100nM) were markedly inhibited. Both PD123319 and CGP42112 did not affect basal CA

output. CGP42112 (15 nM) perfused into an adrenal vein for 90 min time-dependently inhibited the CA secretory responses evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644, cyclopiazonic acid, veratridine, and AngII.

Collectively, these present results suggest that both PD123319 and CGP42112 inhibit the CA secretion evoked by AngII as well as cholinergic (both nicotininc and muscarinic receptors) stimulation and direct membrane depolarization from the perfused rat adrenal medulla. It seems that both drugs have antagonist activity at nicotinic receptors of the perfused rat adrenal medulla. It is also thought that this inhibitory effect of both PD123319 and CGP42112 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 AT_2 receptor blockade. Based on these present data, it is suggested that both PD123319 and CGP42112 have no agonist activity in the perfused model of isolated rat adrenal medulla, and that AT_2 receptors may be involved in the rat adrenomedullary CA secretion.

ABSTRACT

Effects of AT_2-Receptor Antagonist on Catecholamine Release in the Isolated Perfused Rat Adrenal Medulla

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Previously some studies have demonstrated that the CA-releasing effect of AngII is mediated primarily via the activation of AT_2 receptors in adrenal medullary fragments obtained from the rat adrenal gland (Belloni et al. 1998; Mazzocchi et al. 1998), and also in cultured porcine adrenal medullary chromaffin cells (Takekoshi et al., 2001). In contrast, an AT_2 agonist, CGP 42112, has been shown to inhibit CA biosynthesis through a decrease in cGMP production in cultured porcine adrenal medullary cells (Takekoshi et al., 2000). Martineau and his co-workers (1999) have shown that AT_2 receptors play a role in mediating CA secretion by the adrenal medulla of anesthetized dogs in response to AngII receptor agonist administration *in vivo*. Thus, there seems to be some controversy about the effect of AT_2 receptor blockade on the CA secretion from the adrenal gland. The aim of this study therefore was to determine whether PD123319 could influence the CA release from the isolated perfused model of the rat adrenal medulla. PD123319 (5~50 nM) perfused into an adrenal vein for 90 min produced dose- and time-dependent inhibition in 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 PD123319 (15 nM) for 90 min, 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 Na^{+} channels), and angiotensin II (Ang II, 100nM) were markedly inhibited. Both PD123319 and CGP42112 did not affect basal CA output. CGP42112 (15 nM) perfused into an adrenal vein for 90 min time-dependently inhibited the CA secretory responses evoked by ACh, high K^{+}, DMPP, McN-A-343, Bay-K-8644, cyclopiazonic acid, veratridine, and AngII.

Collectively, these present results suggest that both PD123319 and CGP42112 inhibit the CA secretion evoked by AngII as well as cholinergic (both nicotininc and muscarinic receptors) stimulation and direct membrane depolarization from the perfused rat adrenal medulla. It seems that both drugs have antagonist activity at nicotinic receptors of the perfused rat adrenal medulla. It is also thought that this inhibitory effect of both PD123319 and CGP42112 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 AT_2 receptor blockade. Based on these present data, it is suggested that both PD123319 and CGP42112 have no agonist activity in the perfused model of isolated rat adrenal medulla, and that AT_2 receptors may be involved in the rat adrenomedullary CA secretion.

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.

Ardaillou R. Antiotensin II receptors. *J Am Soc Nephrol* 10: S30–S39, 1999.

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.

Balla T, Baukal AJ, Eng S, Catt KJ. Angiotensin II receptor subtypes and biological responses in the adrenal cortex and medulla. *Mol Pharmacol* 40: 401–406, 1991.

Belloni AS, Andreis PG, Macchi V, Gottardo G, Malendowicz LK, Nussdorfer GG. Distribution and functional significance of angiotensin-II AT1- and AT2-receptor subtypes in the rat adrenal gland. *Endocr Res* 24: 1–15, 1998.

Brechler V, Jones PW, Levens NR, De Gasparo M, Bottari SP. Agonistic and antagonistic properties of angiotensin analogs at the AT2 receptor in PC12W cells. *Regul Pept* 44: 207–213, 1993.

Bumpus FM, Catt KJ, Chiu AT, De Gasparo M, Goodfriend T, Husain A, Peach MJ, Taylor DG, Timmermans PBMWM. Nomenclature for angiotensin receptors. *Hypertension* 17: 720–721, 1991.

Bunn SJ, Marley PD. Effects of angiotensin II on cultured, bovine adrenal medullary cells. *Neuropeptides* 13: 121–132, 1989.

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

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.

Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson A, Timmermans PB. Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Commun* 165: 196–203, 1989.

Defaye G, Lecompte S, Chambaz EM, Bottari SP. Stimulation of cortisol production through angiotensin AT2 receptors in bovine fasciculata cells. *Endocr Res* 21: 183–187, 1995.

Dendorfer A, Raasch W, Tempel K, Dominiak P. Interactions between the renin-angiotensin system (RAS) and the sympathetic system. *Basic Res Cardiol* 93 Suppl 2: 24-29, 1998.

Dunn LA, Holz RW. Catecholamine secretion from digitonin-treated adrenal medullary chromaffin cells. *J Biol Chem* 258: 4989-4993, 1983.

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.

de Gasparo M, Catt KJ, Inagami T. Angiotensin receptors. In: The IUPHAR Receptor Compendium. IUPHAR Media Ltd., The Burlington Press, Foxton, Cambridge, UK, p 80–86, 1998.

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

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.

Hano T, Mizukoshi M, Baba A, Nakamura N, Nishio I. Angiotensin II subtype 1 receptor modulates epinephrine release from isolated rat adrenal gland. *Blood Pressure* 5(Suppl): 105–108, 1994.

Heemskerk FM, Saavedra JM. Quantitative autoradiography of angiotensin II AT2 receptors with [125I]CGP 42112. *Brain Res* 677: 29–38, 1995.

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, Strömberg C, Tsutsumi K, Garrido M del R, Torres M, Saavedra JM. Angiotensin II receptor subtypes and phosphoinositide hydrolysis in rat adrenal medulla. *Brain Res Bull* 38: 441–446, 1995.

Kambayashi Y, Bardhan S, Takahashi K, Tsuzuki S, Inui H, Hamakubo T, Inagami T. Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. *J Biol Chem* 268: 24543–24546, 1993.

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.

Lokuta AJ, Cooper C, Gaa ST, Wang HE, Rogers TB. Angiotensin II stimulates the release of phospholipidderived second messengers through multiple receptor subtypes in heart cells. *J Biol Chem* 269: 4832–4838, 1994.

Lu XY, Grove KL, Zhan W, Speth RC. Pharmacological characterization of angiotensin II AT(2) receptor subtype heterogeneity in the rat adrenal cortex and medulla. *Endocr J* 3: 255–261, 1995.

McGehee DS, Role LW. Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu Rev Physiol* 57: 521-546, 1995.

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.

Mazzocchi G, Gottardo G, Macchi V, Andreis PG, Malendowicz LK, Nussdorfer GG. The AT2 receptor-mediated stimulation of adrenal catecholamine release may potentiate the AT1 receptor-mediated aldosterone secretagogue action of angiotensin-II in rats. *Endocr Res* 24: 17–28, 1998.

Mukoyama M, Nakajima M, Horiuchi M, Sasamura H, Pratt RE, Dzau VJ. Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors. *J Biol Chem* 268: 24539–24542, 1993.

Nahmias C, Strosberg AD. The angiotensin AT2 receptor: searching for signal-transduction pathways and physiological function. *Trends Pharmacol Sci* 16: 217–252, 1995.

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.

Pelet C, Mironneau C, Rakotoarisoa L, Neuilly G. Angiotensin II receptor subtypes and contractile responses in portal vein smooth muscle. *Eur J Pharmacol* 279: 15–24, 1995.

Powis DA, O'Brien KJ. Angiotensin II increases catecholamine release from bovine adrenal medulla but does not enhance that evoked by K+ depolarization or by carbachol. *J Neurochem* 57: 1461–1469, 1991.

Sasaoka Tetsumasa, Egi Yasuhiro, Tawa Masashi, Yamamoto Ai, Ohkita Mamoru, Takaoka Masanori, Maruyama Tomoyuki, Akira Toshiaki, Matsumura Yasuo. Angiotensin II Type 2 Receptor-Mediated Inhibition of Norepinephrine Release in Isolated Rat Hearts. *J Cardiovascular Pharmacology* 52: 176-183, 2008.

Schäfer F, Muller AR, Schmid HA, Gerstberger R, Simon E. Angiotensin II receptor subtypes in the duck subfornical organ: an electrophysiological and receptor autoradiographic investigation. *Brain Res* 711: 118–124, 1996.

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.

Stoehr SJ, Smolen JE, Holz RW, Agranoff BW. Inositol trisphosphate mobilizes intracellular calcium in permeabilized adrenal chromaffin cells. *J Neurochem* 46(2): 637-640, 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.

Takekoshi K, Ishii K, Isobe K, Nanmoku T, Kawakami Y, Nakai T. Angiotensin-II subtype 2 receptor agonist (CGP-42112) inhibits catecholamine biosynthesis in cultured porcine adrenal medullary chromaffin cells. *Biochem Biophys Res Commun* 272: 544–550, 2000.

Takekoshi K, Ishii K, Kawakami Y, Isobe K and Nakai T. Activation of angiotensin II subtype 2 receptor induces catecholamine release in an extracellular Ca(2+)-dependent manner through a decrease of cyclic guanosine 3′,5′-monophosphate production in cultured porcine adrenal medullary chromaffin cells. *Endocrinol* 142: 3075–3086, 2001.

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

Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JAM, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 45: 205–251, 1993.

Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Smith RD. New perspectives in angiotensin system control. *J Hum Hypertens* 7 Suppl 2: S19-31, 1993.

Unger T, Chung O, Csikos T, Culman J, Gallinat S, Gohlke P, Hohle S, Meffert S,

Stoll M, Stroth U, Zhu YZ. Angiotensin receptors. *J Hypertens* 14: S95–S103, 1996.

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.

Whitebread S, Mele M, Kamber B, de-Gasparo M. Preliminary biochemical characterization of two angiotensin II receptor subtypes. *Biochem Biophys Res Commun* 163: 284–291, 1989.

Whitebread SE, Tayler V, Bottari SP, Kamber B, de Gasparo M. Radioiodinated CGP 42112A: a novel high affinity and highly selective ligand for the characterization of angiotensin AT2 receptors. *Biochem Biophys Res Commun* 181: 1365–1371, 1991.

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.

Worck RH, Frandsen E, Ibsen H, Petersen JS. AT_1 and AT_2 receptor blockade and epinephrine release during insulin-induced hypoglycemia. *Hyperten* 31(Part 2): 384–390, 1998.

Effects of AT_2-Receptor Antagonist on Catecholamine Release in the Isolated Perfused Rat Adrenal Medulla

By

Bhandary Bidur

A Thesis Submitted to the Department of Medical Science in Partial Fulfilment of the Requirements for the Degree of Master in Medicine (Pharmacology Major) at Graduate School, Chosun University

November, 2009

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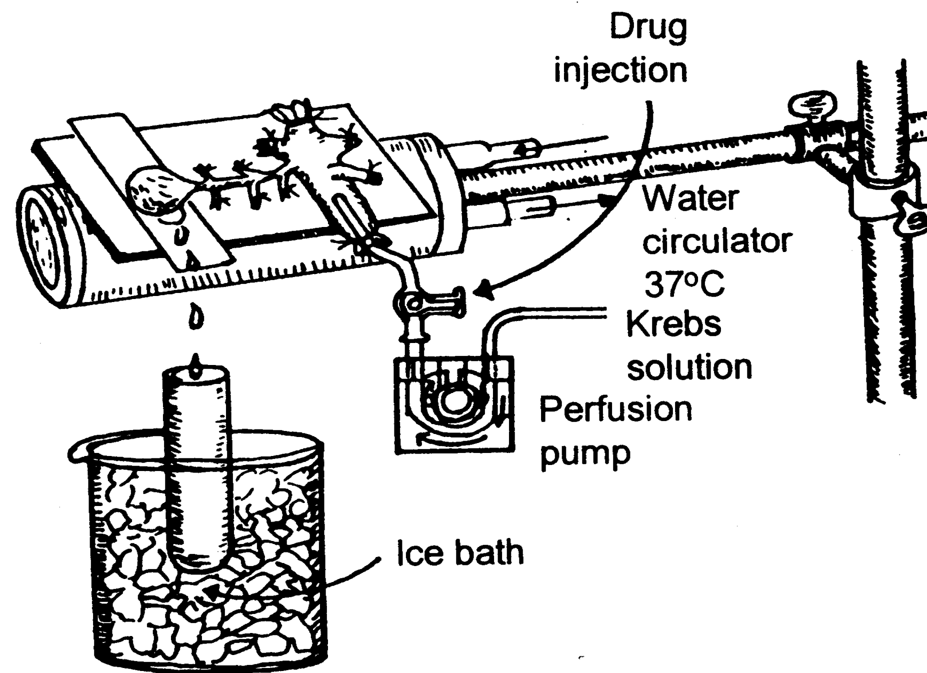

Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamines in the isolated perfused rat adrenal gland.

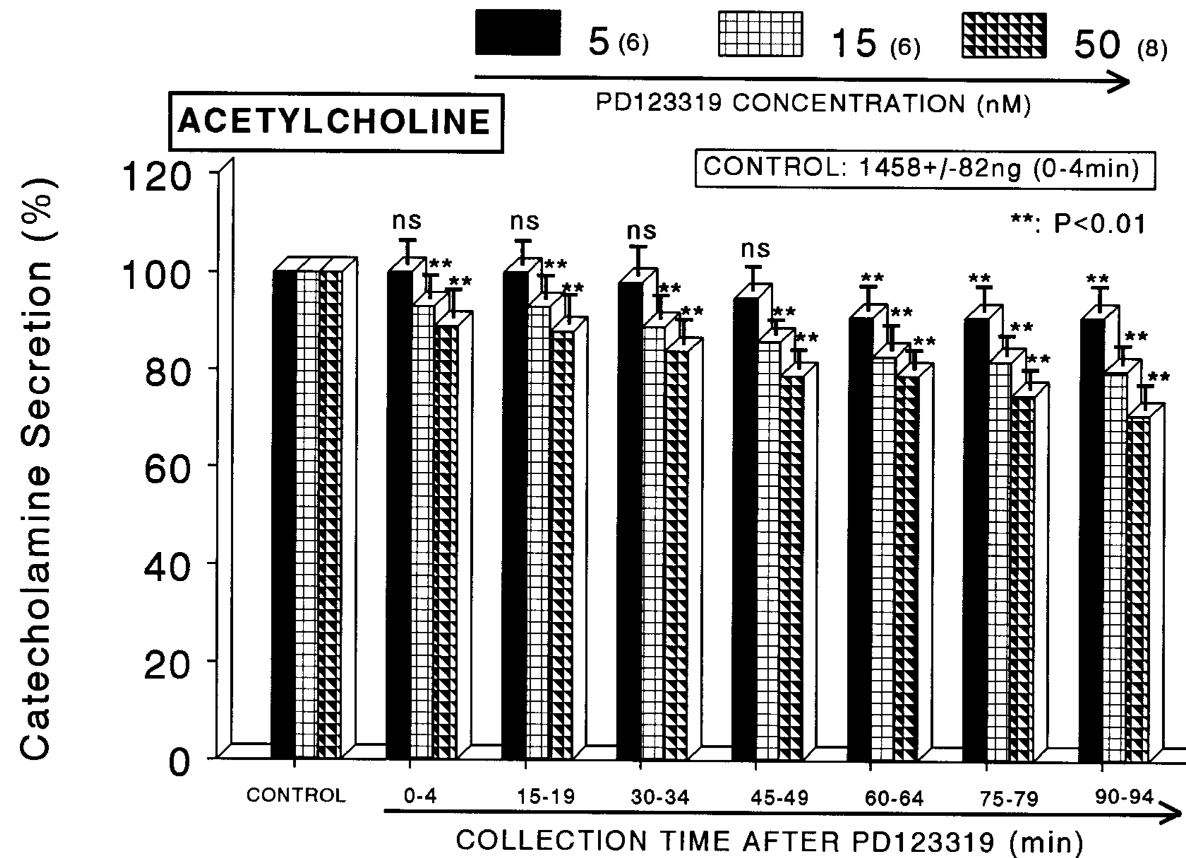

Fig. 2. Dose-dependent effects of PD123319 on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 5, 15, and 50 nM of PD123319 for 90 min as indicated at an arrow mark. 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 PD123319. ACh-induced perfusate was collected for 4 minutes. **: $P < 0.01$. ns; Statistically not significant

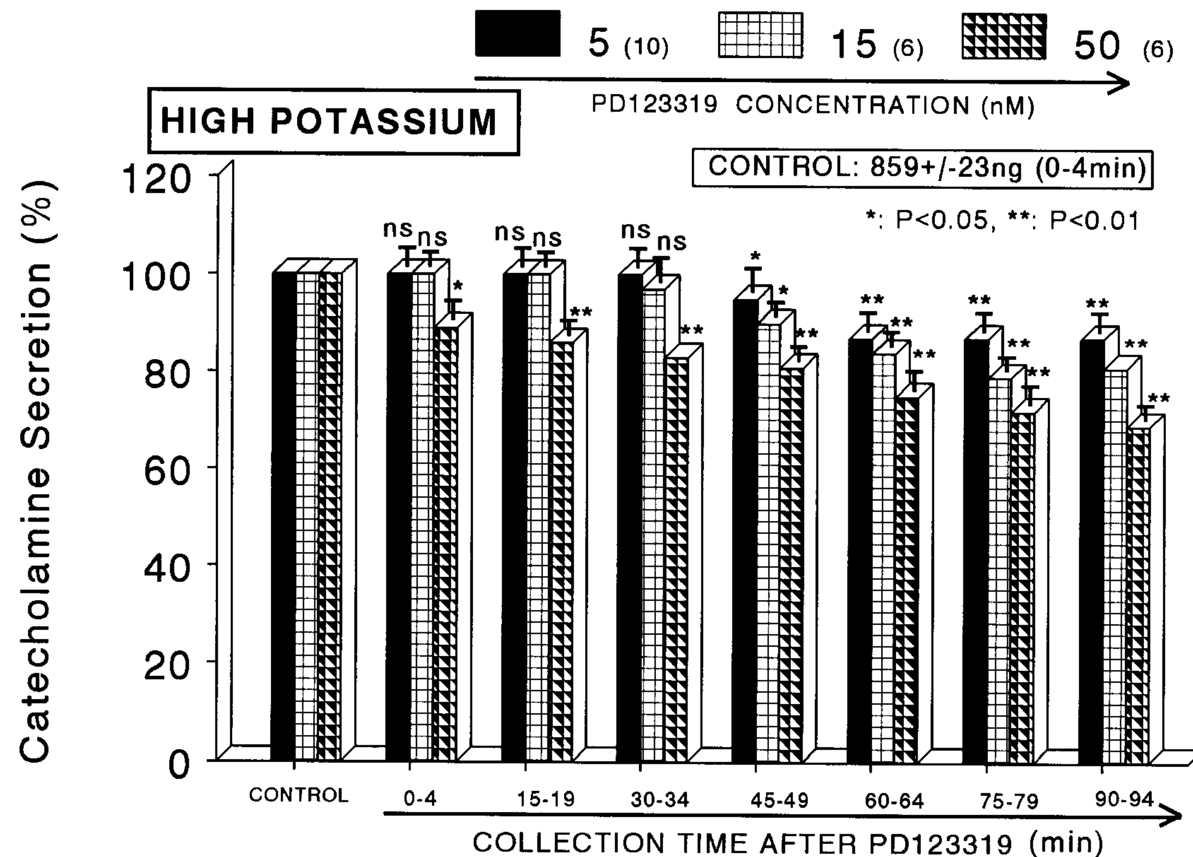

Fig. 3. Dose-dependent effects of PD123319 on the secretory responses of catecholamines (CA) evoked by high K^+ from the isolated perfused rat adrenal glands. CA secretion by a single injection of K^+ (56 mM) was injected in a volume of 0.1 ml at 15 min intervals after preloading with 5, 15, and 50 nM of PD123319 for 90 min. Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of PD123319. K^+-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.

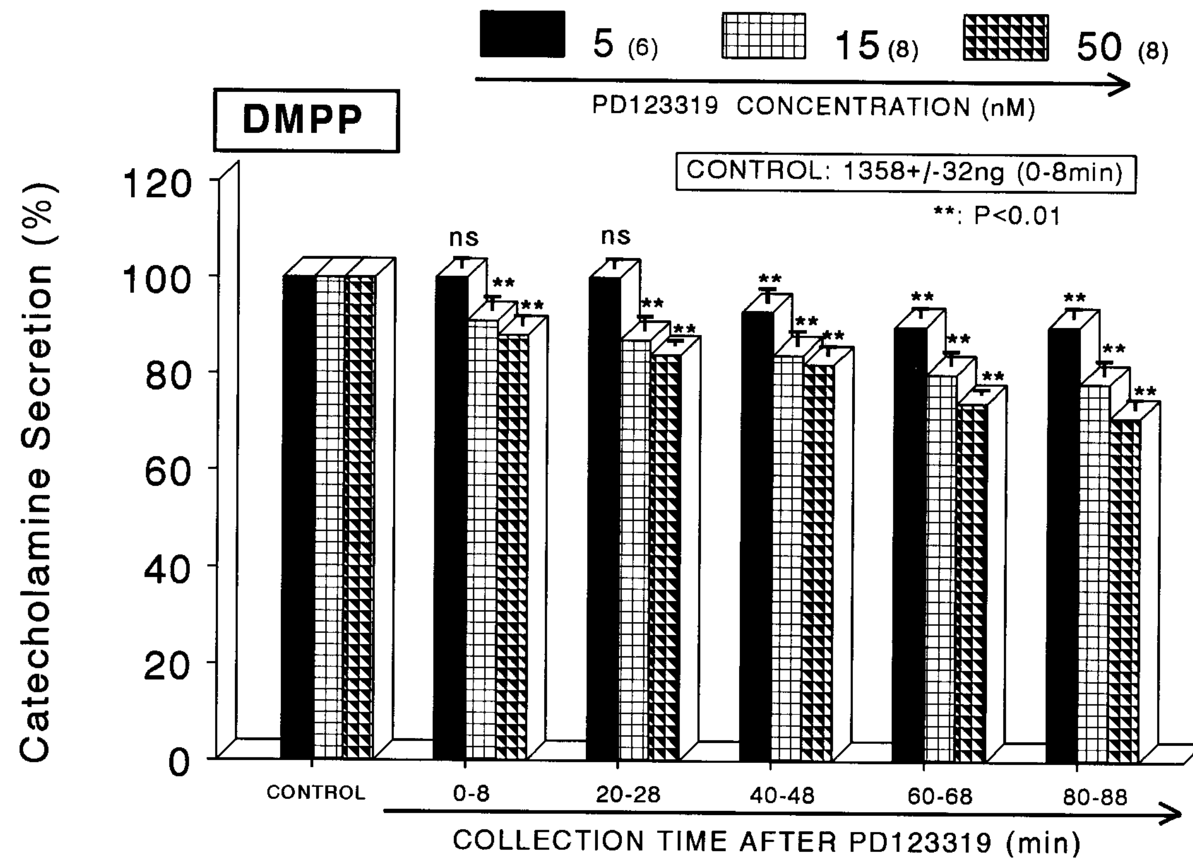

Fig. 4. Dose-dependent effects sof PD123319 on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. CA secretion by the perfusion of DMPP (10^{-4} M) was infused for 2 min at 20 min intervals after preloading with 5, 15, and 50 nM of PD123319 for 90 min. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of PD123319. DMPP-induced perfusate was collected for 8 minutes. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns; Statistically not significant.

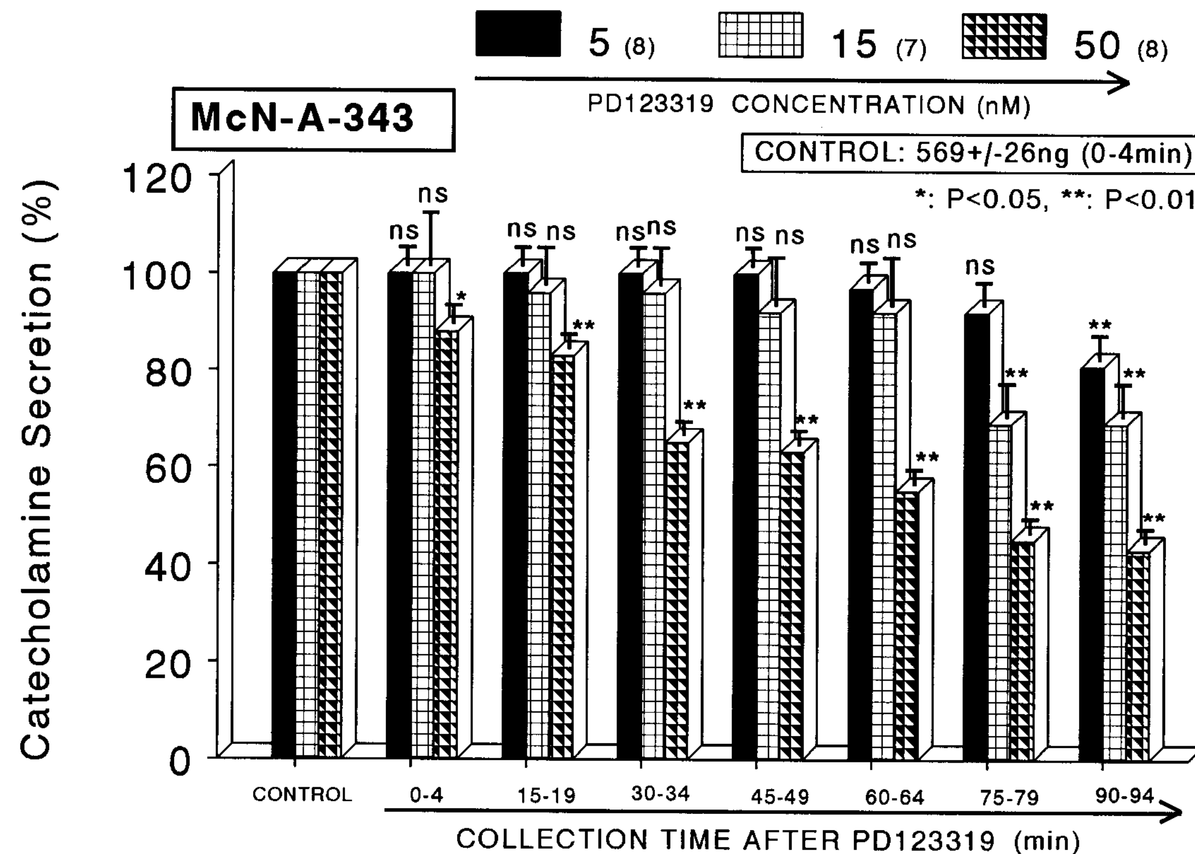

Fig. 5. Dose-dependent effects of PD123319 on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. CA secretion by the perfusion of McN-A-343 (10^{-4} M) was infused for 4 min at 15 min intervals after preloading with 5, 15, and 50 nM of PD123319 for 90 min. Statistical difference was obtained by comparing the corresponding control with each concentration-pretreated group of PD123319. 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.

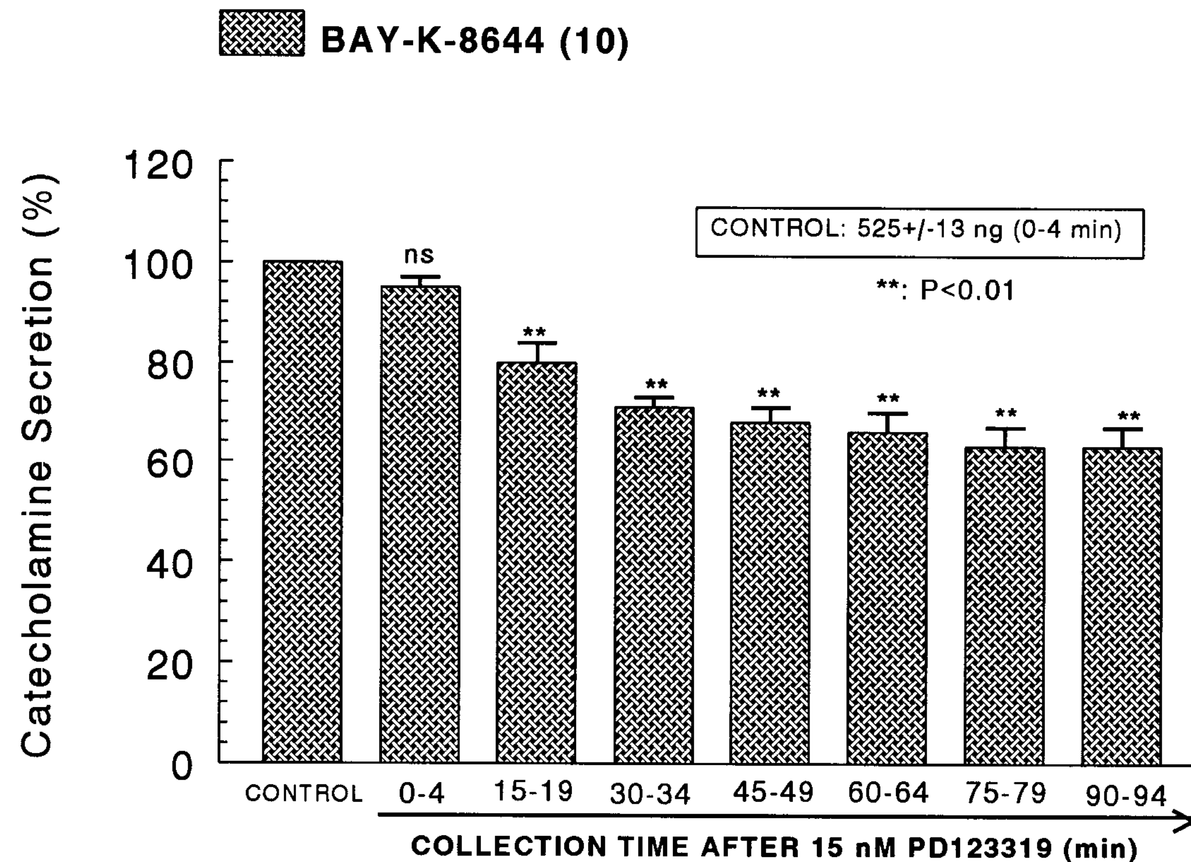

Fig. 6. Time-course effects of PD123319 on CA release evoked by Bay-K-8644 from the rat adrenal glands. Bay-K-8644 (10^{-5} M) was perfused into an adrenal vein for 4 min at 15 min intervals after preloading with PD123319 (15 nM) for 90 min. Statistical difference was obtained by comparing the corresponding control with each period after pretreatment with PD123319. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns; Statistically not significant.

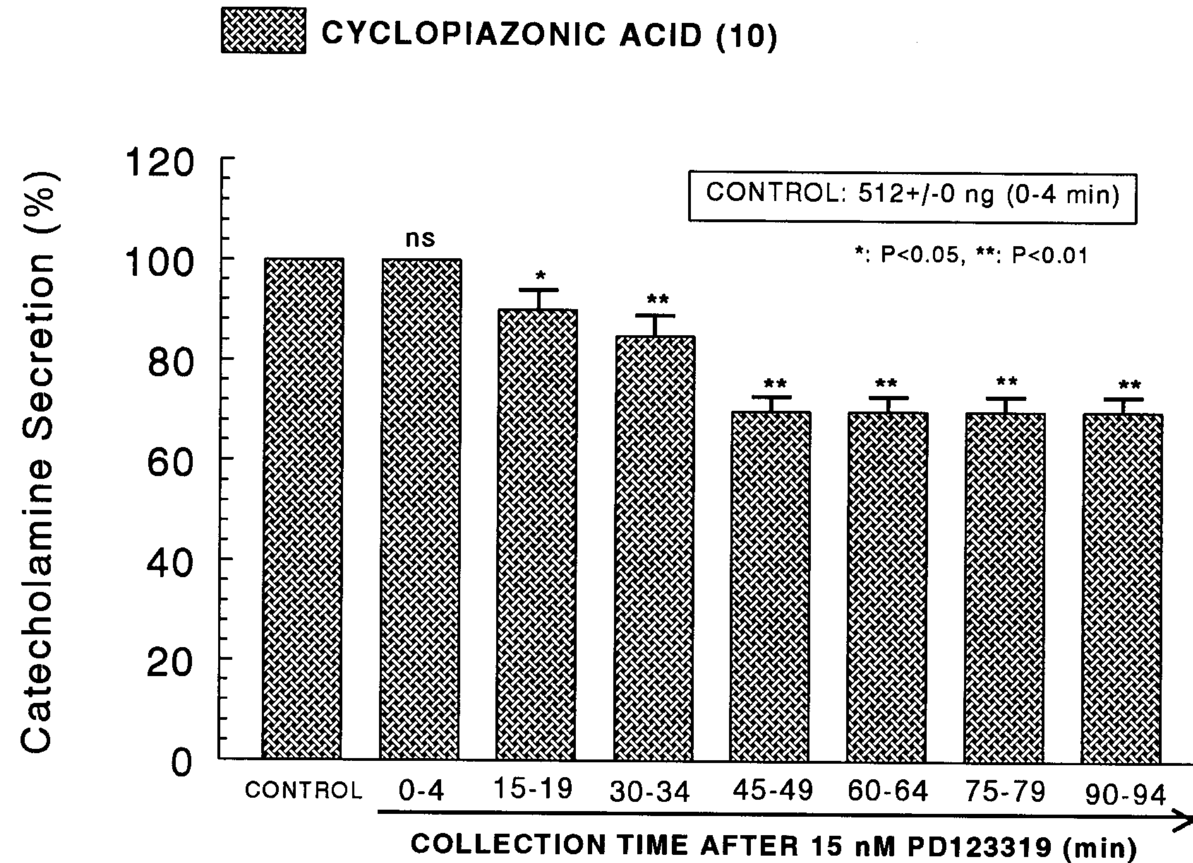

Fig. 7. Time-course effects of PD123319 on CA release evoked by cyclopiazonic acid from the rat adrenal glands. Cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with PD123319 (15 nM) for 90 min. Statistical difference was obtained by comparing the corresponding control with each period after pretreatment with PD123319. Other legends are the same as in Fig. 2. . *: $P < 0.05$, **: $P < 0.01$. ns; Statistically not significant.

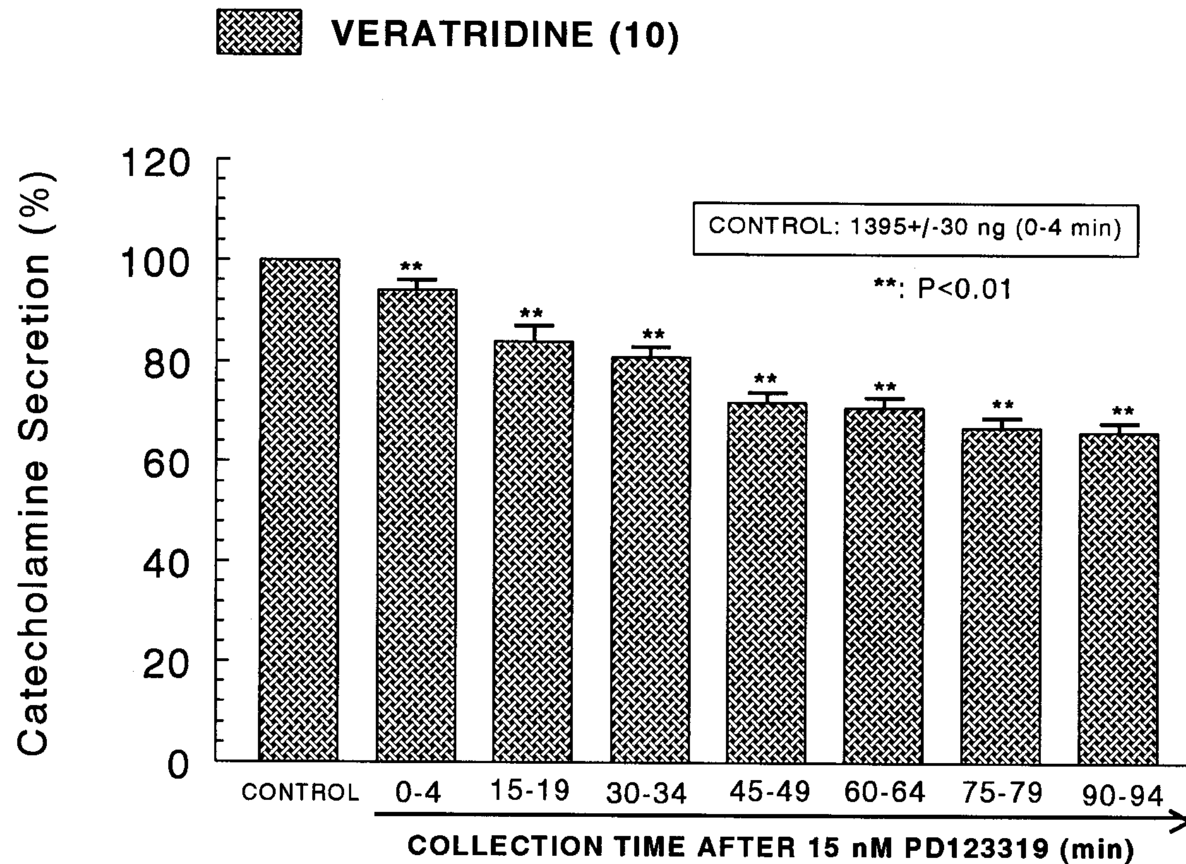

Fig. 8. Time-course effects of PD123319 on the CA release evoked by veratridine from the rat adrenal glands. Veratridine (10^{-4} M) was perfused into an adrenal vein for 4 min at 15 min intervals after preloading with PD123319 (15 nM) for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$.

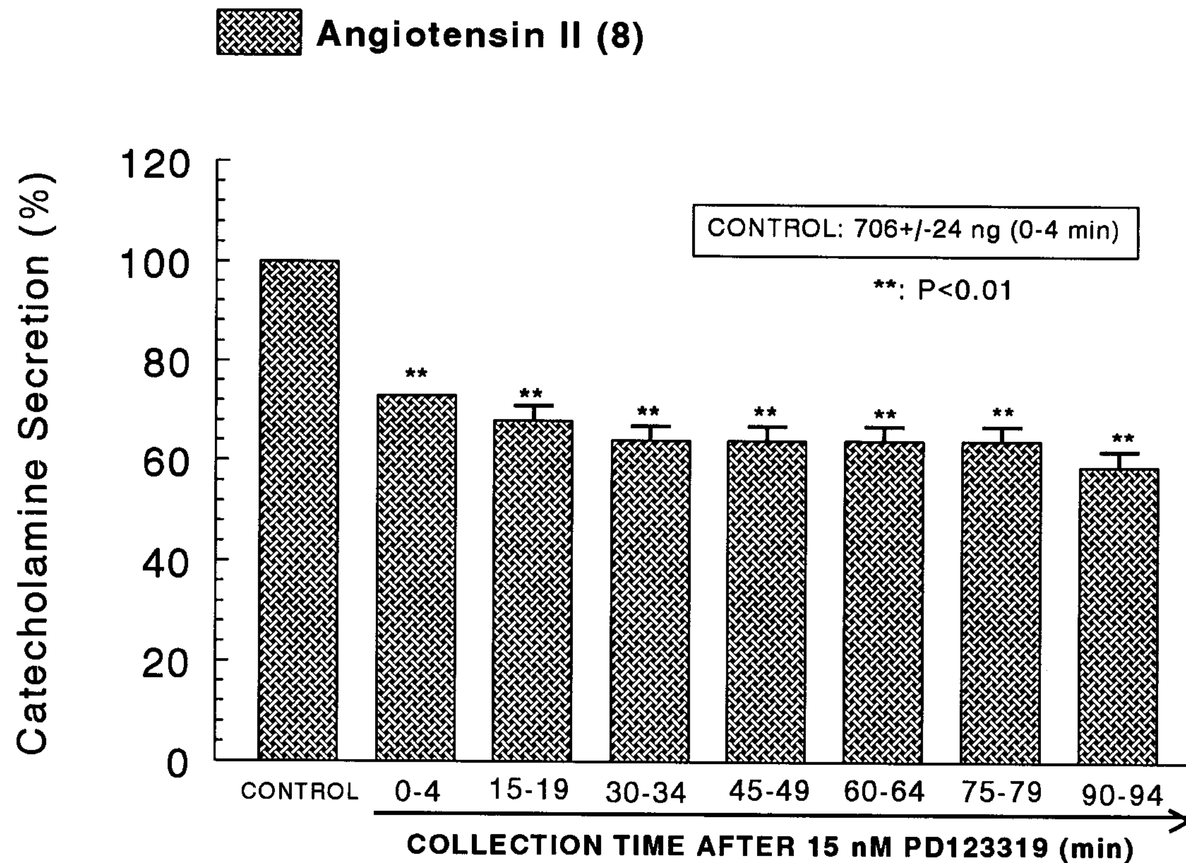

Fig. 9. Time-course effects of PD123319 on the CA release evoked by angoitensin II from the rat adrenal glands. Angotensin II (10^{-6} M) was perfused into an adrenal vein for 1 min at 15 min intervals after preloading with PD123319 (15 nM) for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$.

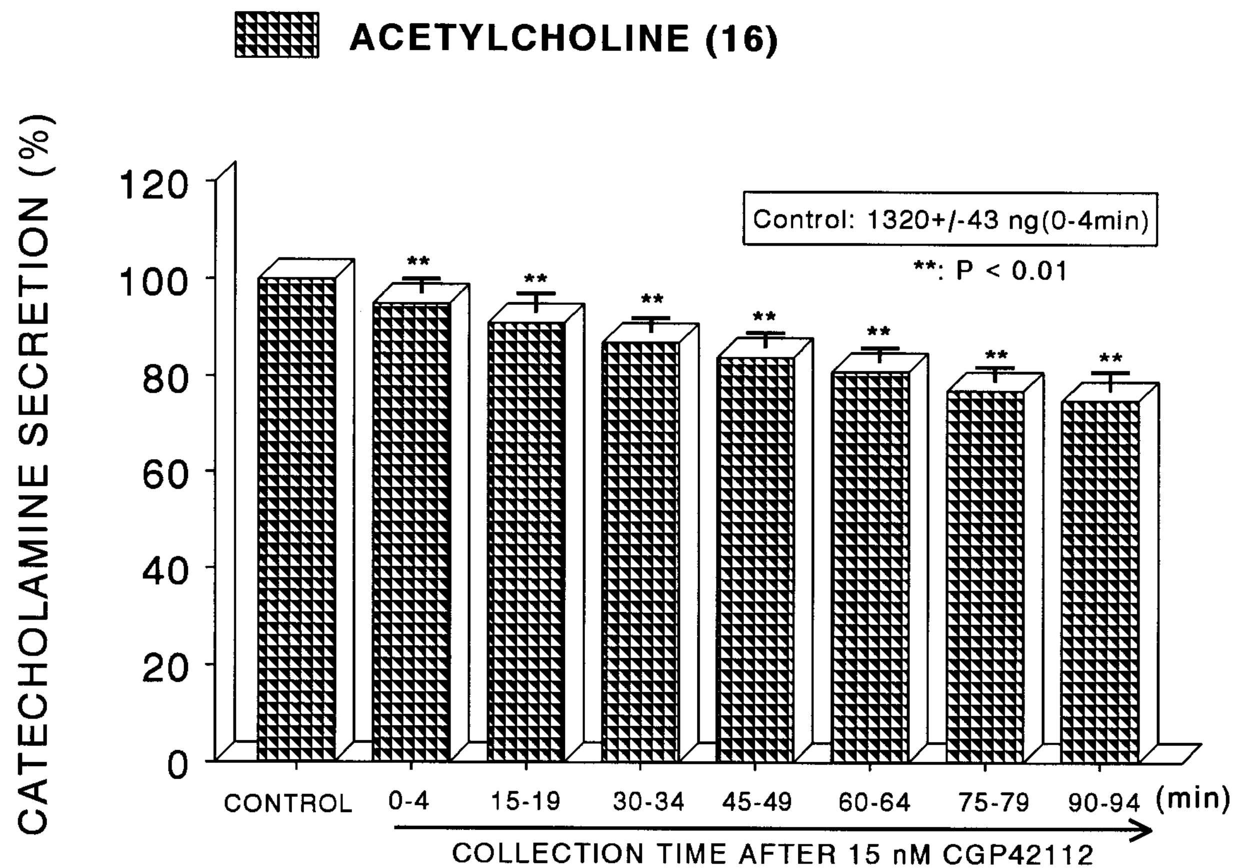

Fig. 10. Time-course effects of CGP42112 on the CA release evoked by acetylcholine (ACh) from the rat adrenal glands. CA secretion by a single injection of ACh (5.32 x 10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with CGP42112 (15 nM) for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$.

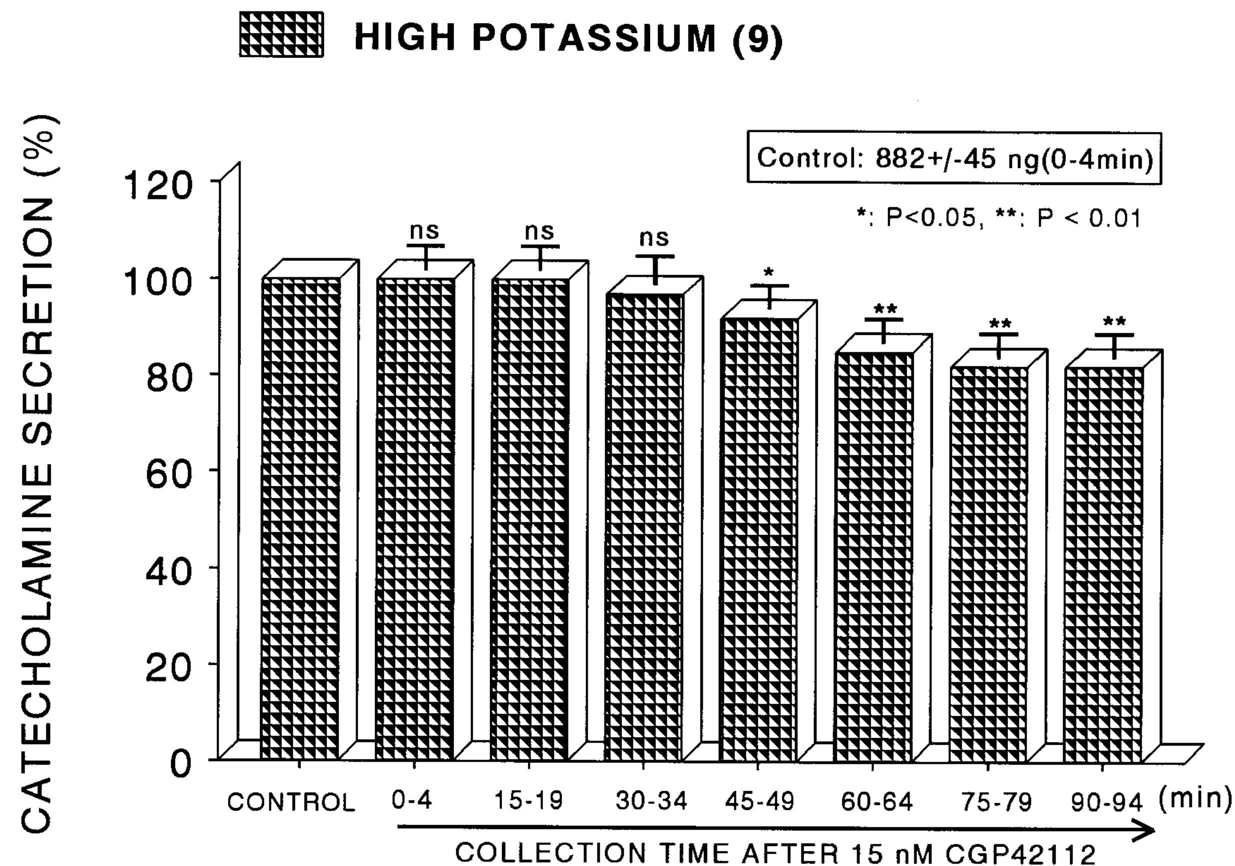

Fig. 11. Time-course effects of CGP42112 on the secretory responses of catecholamines (CA) evoked by high K^+ from the isolated perfused rat adrenal glands. CA secretion by a single injection of K^+ (56 mM) was injected in a volume of 0.1 ml at 15 min intervals after preloading with 15 nM of CGP42112 for 90 min. Other legends are the same as in Fig. 2. *: $P < 0.05$, **: $P < 0.01$. ns; Statistically not significant.

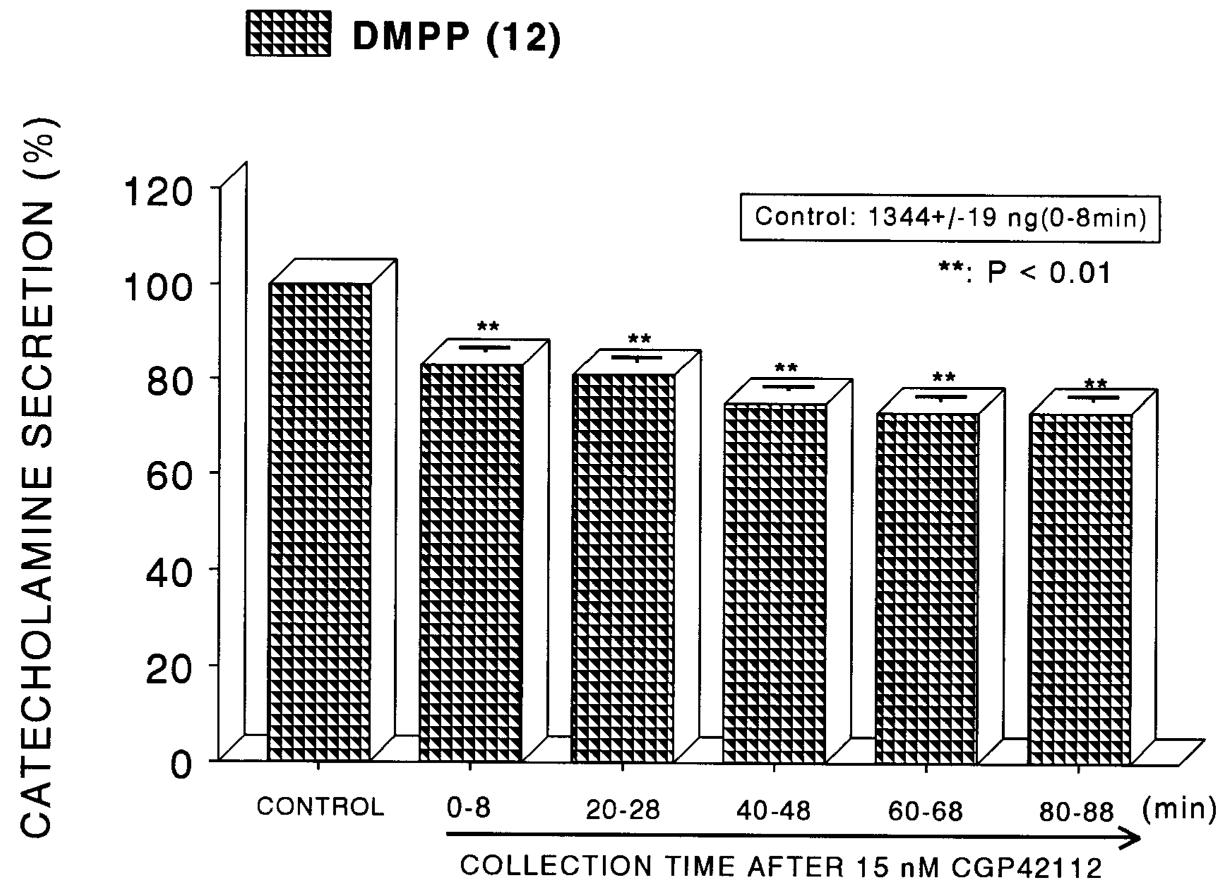

Fig. 12. Time-course effects sof CGP42112 on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. CA secretion by the perfusion of DMPP (10^{-4} M) was infused for 2 min at 20 min intervals after preloading with 15 nM of CGP42112 for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns; Statistically not significant.

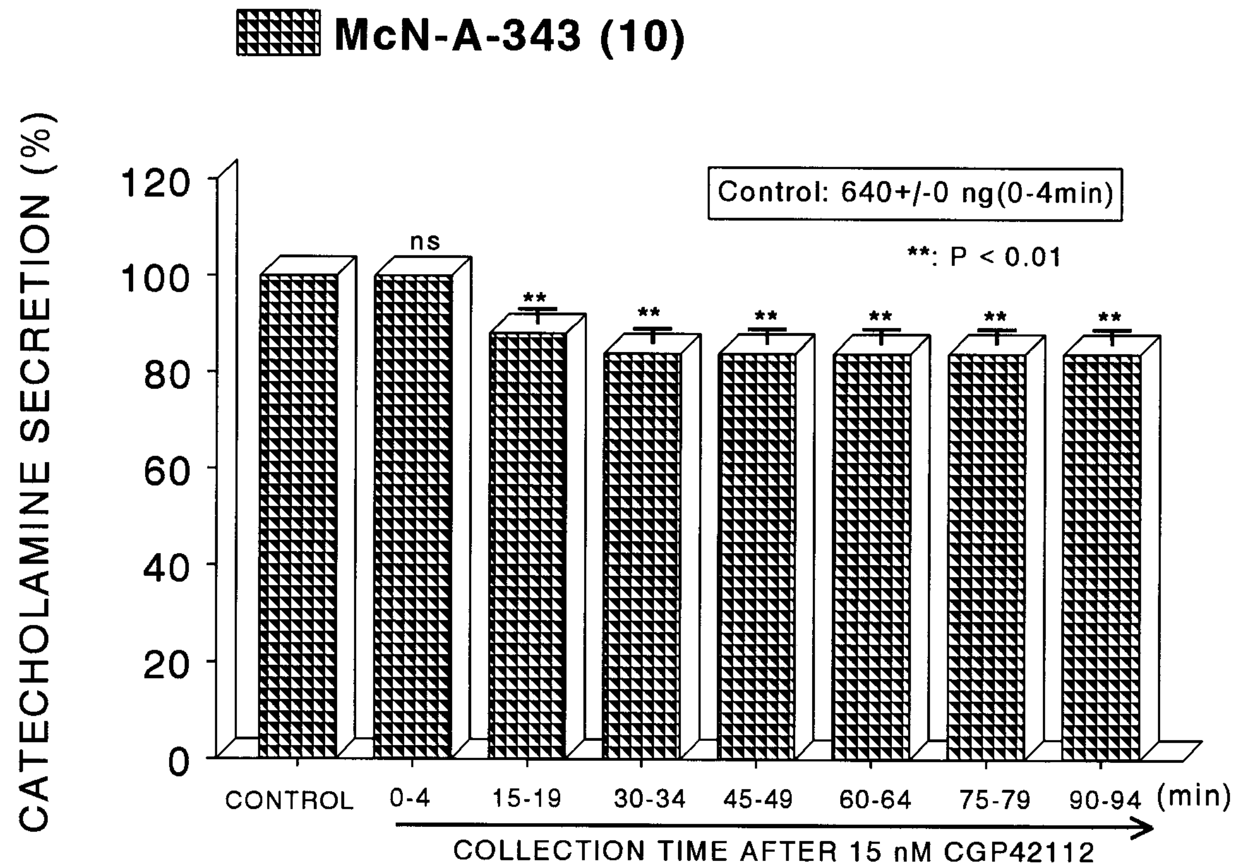

Fig. 13. Time-course effects of CGP42112 on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. CA secretion by the perfusion of McN-A-343 (10^{-4} M) was infused for 4 min at 15 min intervals after preloading with 15 nM of CGP42112 for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns; Statistically not significant.

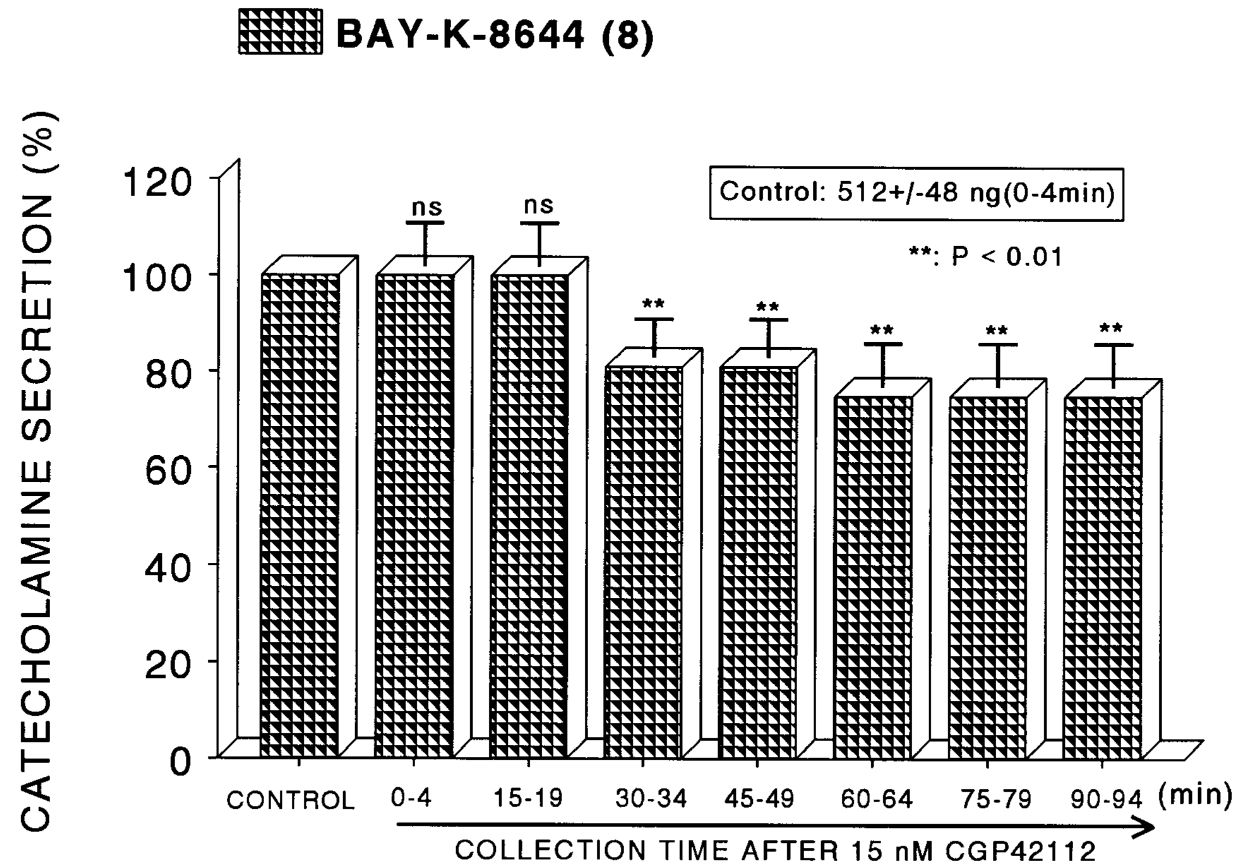

Fig. 14. Time-course effects of CGP42112 on CA release evoked by Bay-K-8644 from the rat adrenal glands. Bay-K-8644 (10^{-5} M) was perfused into an adrenal vein for 4 min at 15 min intervals after preloading with CGP42112 (15 nM) for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$. ns; Statistically not significant.

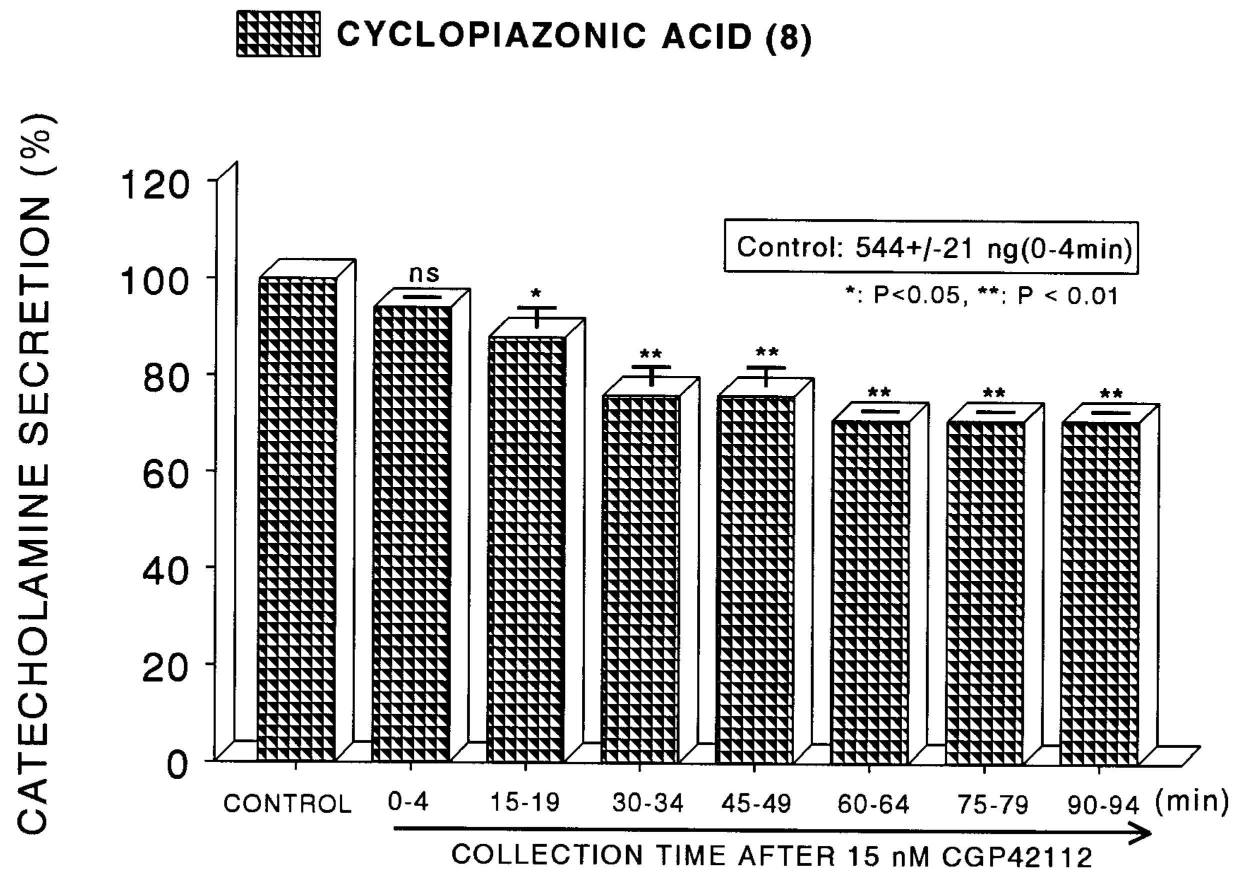

Fig. 15. Time-course effects of CGP42112 on CA release evoked by cyclopiazonic acid from the rat adrenal glands. Cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with CGP42112 (15 nM) for 90 min. Other legends are the same as in Fig. 2. . *: $P < 0.05$, **: $P < 0.01$. ns; Statistically not significant.

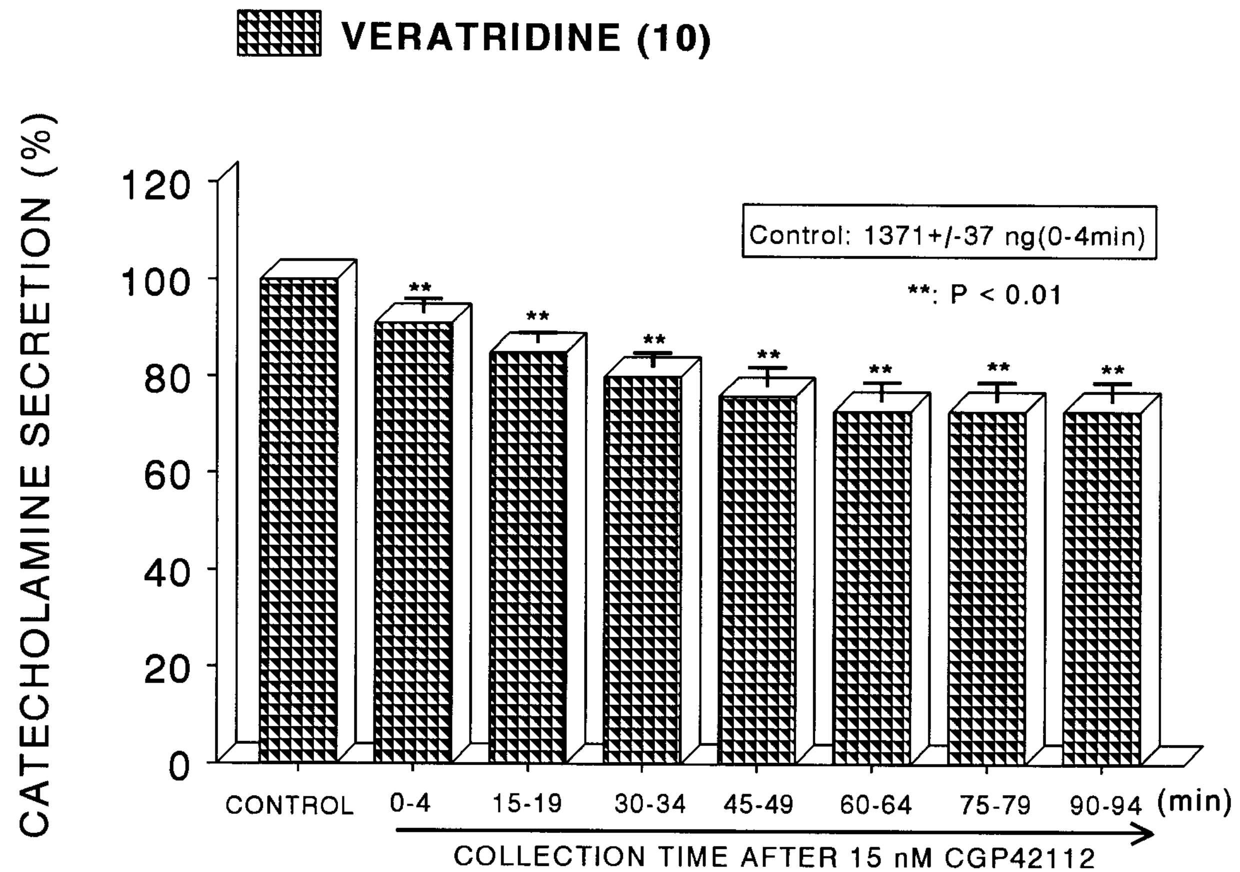

Fig. 16. Time-course effects of CGP42112 on the CA release evoked by veratridine from the rat adrenal glands. Veratridine (10^{-4} M) was perfused into an adrenal vein for 4 min at 15 min intervals after preloading with CGP42112 (15 nM) for 90 min. Other legends are the same as in Fig. 2. **: $P < 0.01$.

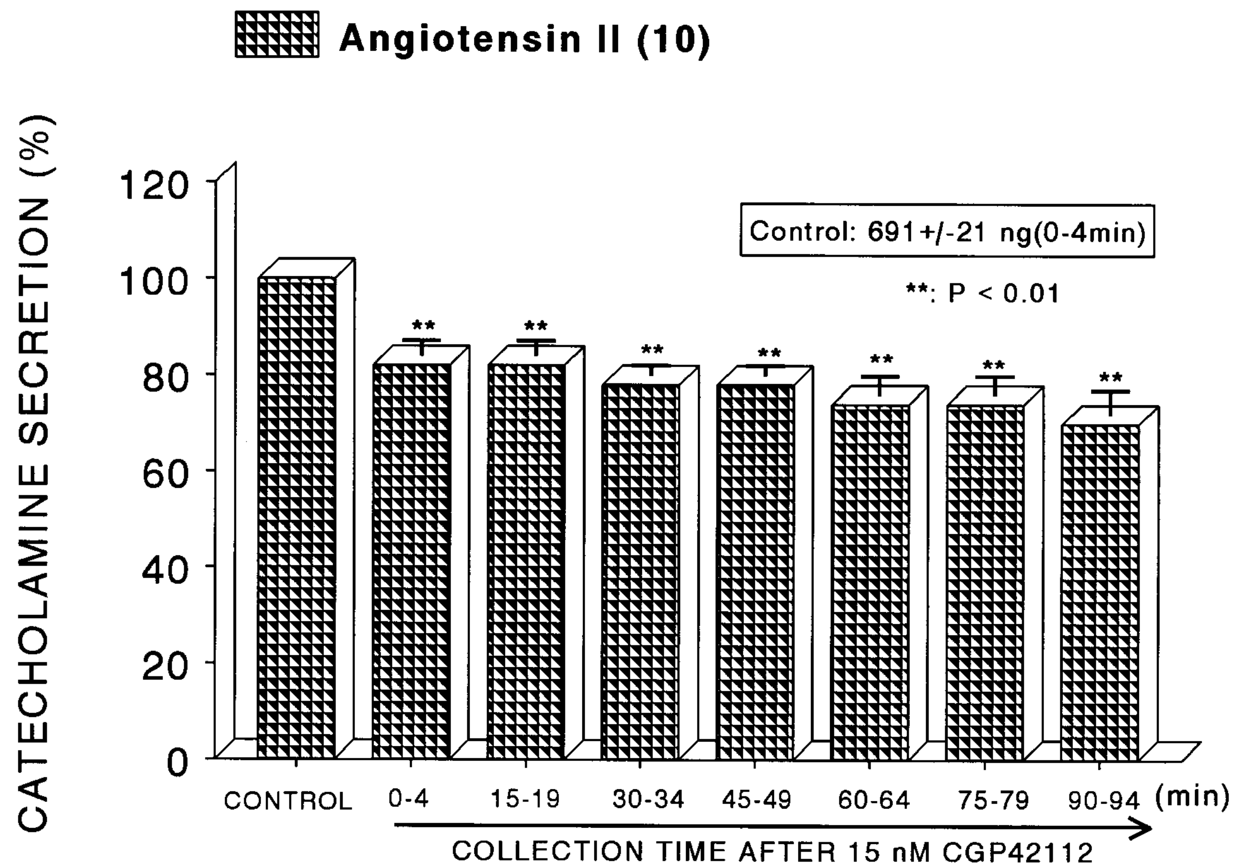

Fig. 17. Time-course effects of CGP42112 on the CA release evoked by angoitensin II from the rat adrenal glands. Angotensin II (10^{-6} M) was perfused into an adrenal vein for 1 min at 15 min intervals after preloading with CGP42112 (15 nM) for 90 min, respectively. Other legends are the same as in Fig. 2. **: $P < 0.01$.

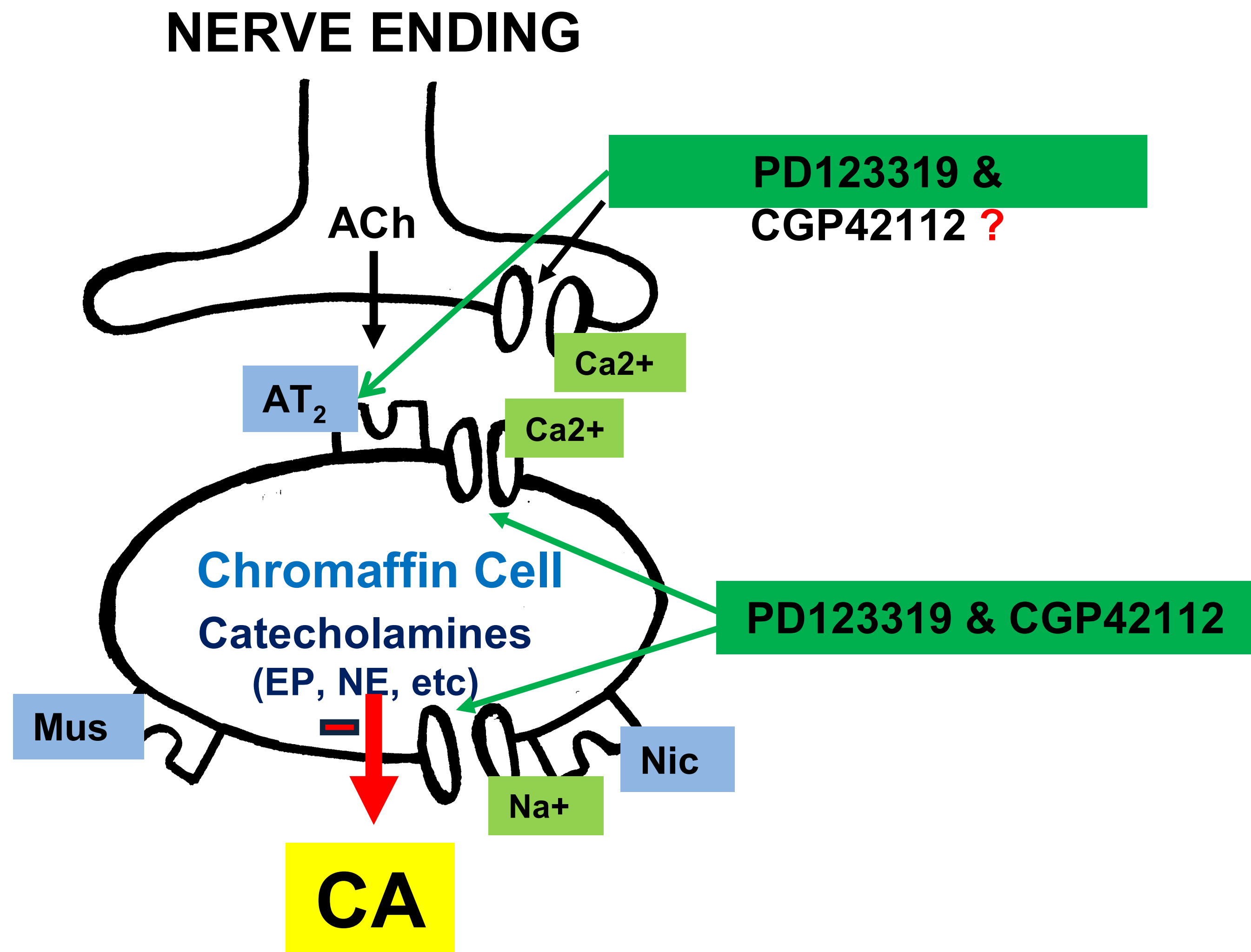

Fig. 18. Schematic diagram of possible action site of PD123319 and CGP42112 at the cholinergic nerve ending-chromaffin cell synapse in the the rat adrenal gland.

저작물 이용 허락서

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논문제목	한글 : **흰쥐 적출부신수질에서 AT_2 수용체 길항제가 카테콜아민 유리에 미치는 영향** 영어 : Effects of AT_2-Receptor Antagonist on Catecholamine Release in the Isolated 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 편집ㆍ형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포ㆍ전송된 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 전송ㆍ출력을 허락함.

동의여부 : 동의(0) 반대( )

2009년 11월 일

저작자: Bhandary Bidur (서명 또는 인)

조선대학교 총장 귀하