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Roles of Dopaminergic D_1 and D_2 Receptors in Catecholamine Release from the Rat Adrenal Medulla

조선대학교 대학원

의학과

백영주

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흰쥐 부신수질에서 카테콜아민 유리에 대한
도파민 D_1 및 D_2 수용체의 역할

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<국문 초록>

흰쥐 부신수질에서 카테콜아민 유리에 대한 도파민 D_1 및 D_2 수용체의 역할

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Dahmer 및 Senogles (1996)는 선택적인 도파민 D_1 수용체 작동제인 CI-APB 와 SKF-38393 가 DMPP 에 의한 카테콜아민(catecholamines, CA)분비 작용을 농도의존적으로 억제한다고 하였다. 그러나 이와는 대조적으로, 개의 부신수질에서 카테콜아민(CA) 유리에 대한 도파민의 억제작용은 도파민 D_2 수용체에 의해서 매개되며 D_1 수용체는 관여하지 않는 것으로 알려져 있다 (Damas-Mischel 등, 1999). 또한 소의 일차 배양 크롬친화세포에서도 도파민 D_2 수용체의 CA 분비 억제작용은 도파민 D_1 수용체와의 상호작용에 의하지 않는 것으로 알려져 있다 (Bigornia 등, 1988; 1990). 따라서 본 연구의 목적은 선택적인 도파민 D_1 수용체 작동제인 SKF81297 과 선택적인 도파민 D_2 수용체 작동제인 R(-)-TNPA 가 흰쥐 적출 관류부신에서 콜린성 흥분작용과 막 탈분극에 의한 CA 분비 억제작용을 비교하여 도파민수용체의 역할을 확립코자 본 연구를 시행하여 다음과 같은 결과를 얻었다.

SKF81297 (30 μM) 및 R(-)-TNPA(30 μM)을 각각 부신정맥내로 60 분간 관류시 비교적 시간의존적으로 ACh (5.32 x 10^{-3} M), DMPP (10^{-4} M), McN-A-343 (10^{-4} M), high K^{+} (5.6 x 10^{-2} M), Bay-K-8644 (10 μM) 및 cyclopiazonic acid (10 μM) 에 의한 CA 분비반응을 현저하게 억제하였다. ACh, 고칼륨, DMPP, McN-A-343, Bay-K-8644 및 cyclopiazonic acid 에 의한 CA 유리작용에 대한 억제작용의 효력의 크기는 SKF81297>R(-)-TNPA 순이었다. 그러나, 선택적인 도파민 D_1 수용체 길항제인 R(+)-SCH23390(3 uM) 과 선택적인 도파민 D_2 수용체 길항제인 S(-)-raclopride(3 uM)을 부신정맥내로 60 분간 관류하였을때, ACh, 고칼륨, DMPP, McN-A-343, Bay-K-8644 및 cyclopiazonic acid 에 의한 CA 분비반응을 처음 4 분 동안만 증강시켰다. 고칼륨, McN-A-343 및 cyclopiazonic acid 에 의한 CA 유리작용에 대한 억제작용의 효력의 크기는 R(+)-SCH23390> S(-)-raclopride 순이었다. 또한, ACh, DMPP, 및 Bay-K-8644 에 의한 CA 유리작용에 대한 억제작용의 효력의 크기는 S(-)-raclopride> R(+)-SCH23390 순이었다. SKF81297 및 R(-)-TNPA, R(+)-SCH23390 및 S(-)-raclopride 자체는 기초 CA 분비량에 영향을 미치지 않았다.

이와 같은 연구결과를 종합하여 보면, 흰쥐 적출 관류 부신수질에서 SKF81297 과 R(-)-TNPA 는 각각 콜린성(니코틴 및 무스카린 수용체) 흥분작용 및 막탈분극에 의한 CA 분비작용에 대하여 억제작용을 나타내었으나. R(+)-SCH23390 및 S(-)-raclopride 는 촉진작용을 나타내었다. 이러한 SKF81297 과 R(-)-TNPA 의 억제작용은 흰쥐 적출 부신수질의 크롬친화세포에 있는 각각 도파민 D_1 및 D_2 수용체의 흥분에 기인하는 반면에

R(+)-SCH23390 및 S(-)-raclopride 의 CA 분비 항진작용은 각각 도파민 D_1 및 D_2 수용체의 차단에 의해서 매개되며, 이와 같은 작용은 세포내외의 칼슘이동에 연관성이 있는것으로 생각된다. 따라서, 기존의 잘 알려진 도파민 D_2 수용체외에 D_1 수용체의 존재가 흰쥐부신수질의 카테콜아민 유리작용을 조절하는데 있어서 중요한 역할을 담당하는 것으로 사료된다.

I. INTRODUCTION

At many cells that undergo exocytosis, autoreceptors play an important regulatory role. For example, at most neurons autoreceptor activation inhibits further release. Autoreceptors on dopaminergic neurons are D2-like (the D2 receptor class is composed of the D_2, D_3, and D_4 dopaminergic receptors), and dopamine autoreceptor effects are absent in mice with a genetic deletion of the D_2 receptor (Benoit-Marand et al., 2001). Similar autoreceptor control has been shown for norepinephrine (Starke, 2001). Inhibitory autoreceptor regulation has been reported at bovine chromaffin cells from the adrenal gland. Early investigations, prompted by the phylogenetic relationship between chromaffin cells and sympathetic neurons that are well established to have autoreceptors, determined that autoreceptors on bovine chromaffin cells had properties that pharmacologically resemble dopaminergic receptors (Gonzalez et al., 1986; Artalejo et al., 1985), and their activation inhibited release. Subsequent research showed that D2 dopamine receptors are located on bovine chromaffin cells and their activation inhibited release (Bigornia et al., 1990). In chromaffin cells, inhibition of secretion of catecholamines (CA) by dopamine agonists has been attributed to D2 dopamine receptors based on the reversal of inhibition by D2-selective antagonists such as haloperidol and butaclamol (Artalejo et al., 1985; Bigornia et al., 1988). In addition, binding sites for tritiated antagonists selective for the D2 family of dopamine receptors were identified on these cells (Gonzalez et al., 1986; Lyon et al., 1987; Quik et al., 1987). At the time, these data were interpreted as evidence that D2 dopamine receptors on the cells

inhibited the CA release. Furthermore, results obtained *in vivo* mostly evidence an inhibitory influence of peripheral dopamine receptors on the CA release from adrenals. In anaesthetized and vagotomized dogs with electrically stimulated splanchnic nerves, the dopamine D2-like receptor agonist, quinpirole, had an inhibitory effect, while the dopamine D2-like receptor antagonist, domperidone, had a stimulatory effect on noradrenaline and adrenaline release (Foucart et al., 1988). Stimulation of noradrenaline and adrenaline release was induced in humans by domperidone during exercise (Mercuro et al., 1988; Mannelli et al., 1988) or after glucagon stimulation (Mannelli et al., 1990).

However, experiments performed on conscious rats (Nagahama et al., 1986; Regunathan et al., 1989) indicate the opposite effects, i.e. that dopamine receptor agonists stimulate adrenaline release from the adrenals, suggesting the possibility of species differences. Previous results show that the stimulation of dopamine D2-like receptors, probably peripherallly located, increases synthesis of the CA in the rat adrenal glands (Kujacic et al., 1990,1991; Kujacic and Carlsson, 1993). Kujacic and his co-workers (1995) also demonstrated the presence of peripherally located dopamine D_2-like receptors, capable of acutely stimulating not only the CA synthesis, but also the release of adrenaline from adrenals in the conscious rat.

On the other hand, Huettl et al. (1991), in experiments on bovine chromaffin cell culture, ruled out the presence of functional dopamine D2 receptors of the classical type in the modulation of the CA release. Moreover, it has also been reported that peripheral D_2 receptors are not involved in the control of CA release from the adrenal medulla under in vitro conditions in dogs (Damase-Michel, et al., 1990).

A D1-like receptor (the D1 receptor class is composed of the D_1 and D_5 dopaminergic receptors) has also been identified on bovine chromaffin cells (Artalejo et al., 1990). Activation of this receptor facilitates an inward Ca^{2+} current that could promote exocytosis (Artalejo et al., 1990). More recently, the D1 agonist, SKF-38393, enhanced the number of exocytotic events as did prior exposure of the cell to epinephrine from bovine adrenal chromaffin cells (Villanueva and Wightman, 2007).

However, in contrast to these findings, Dahmer and Senogles (1996) have observed that the D_1-selective agonists 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (CI-APB) and SKF-38393 inhibit DMPP-stimulated CA secretion in a concentration-dependent manner. Moreover, in bovine adrenal chromaffin cells, D_1-selective agonists are found to inhibit secretagogue-stimulated Na^+ uptake in a cyclic AMP-independent manner (Dahmer and Senogles, 1996).

Thus, it is clear that there are many controversial reports on the modulatory effect of dopaminergic subtype receptors in the CA release from the adrenal medulla. The first aim of the present study is to investigate whether both dopaminergic D1 and D2 receptors exist on the rat adrenomedullary chromaffin cells. The second of the present study is to investigate whether the activation of both dopaminergic D1 and D2 receptors can modify the release of CA from the perfused model of the adrenal gland, and to establish the its mechanism of action. Therefore, the present study was carried out to compare their effects of SKF81297, a selective agonist of dopaminergic D_1 receptors with S(-)TNPA, a selective agonist of dopaminergic D2 receptors, on the CA secretion evoked by

cholinergic stimulation and membrane depolarization from the isolated perfused model of the rat adrenal gland, along with the responses to R(+)-SCH23390, a selective antagonist of dopaminergic D_1 receptors, and S(-)-Racropride, a selective antagonist of dopaminergic D2 receptors, and to establish the its mechanism of action.

II. MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 300 grams, were anesthetized with thiopental sodium (40 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.

Perfusion of adrenal gland

The adrenal glands were perfused by means of ISCO pump (WIZ Co.) 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 DMPP (10^{-4} M) for 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), 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, secretory responses to ACh, KCl, McN-A-343, 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 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 SKF81297 or R-(-)-TNPA on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing SKF81297 or R(+)-SCH23390 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 SKF81297 or R-(-)-TNPA, 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 (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 (S.E.M.). 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: 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetra hydro-1H-3-benzazepine (SKF81297), (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl -5-phenyl-1H-benzazepine-7-ol [R(+)-SCH23390], R-(-)-2, 10, 11-trihydroxy -N-propyl-noraporphine hydrobromide (R-(-)-TNPA), S-(-)-raclopride (+)-tartrate salt, 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) (Sigma Chemical Co., U.S.A.), and cyclopiazonic acd, (3-(m-cholro-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 used are expressed in terms of molar base.

III. RESULTS

Effects of SKF81297 and R-(-)-TNPA on acetylcholine-evoked catecholamine (CA) secretion from the perfused rat adrenal glands

After the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 21 ± 2 ng (0-2min, n=9). Previously, it has been found that D2 dopamine receptors are located on bovine chromaffin cells and their activation inhibited release (Bigornia et al., 1990), and also that the D_1-selective agonists CI-APB and SKF-38393 inhibit DMPP-stimulated CA secretion in a concentration-dependent manner (Dahmer and Senogles, 1996), Therefore, it was decided initially to examine the effects of SKF81297 and R-(-)-TNPA on ACh-evoked CA secretion from the isolated perfused rat adrenal glands. ACh was given at 15 min-intervals. SKF81297 or R-(-)-TNPA were present for 60 min including stimulation with ACh, respectively.

In the present study, it was found that SKF81297 and R-(-)-TNPA themselves did not produce any effect on the spontaneous CA release (data not shown). When ACh (5.32×10^{-3} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 365±61 ng (0-4 min). However, after the simultaneous perfusion with SKF81297 (3×10^{-5} M), ACh-stimulated CA secretion was relatively time-dependently inhibited to 47.9 % of the control release (100%) from 5 adrenal glands as shown in Fig. 2. Also, in the presence of R-(-)-TNPA (3×10^{-5} M), a selective dopamine D2 receptor agonist, for 60 min, ACh-evoked CA secretory response was inhibited to 56% of the control release

(Fig. 2).

Effects of SKF81297 and R-(-)-TNPA on high potassium-evoked CA secretion from the perfused rat adrenal glands

Also, it has been found that direct membrane-depolarizing agent like KCl stimulates sharply CA secretion. High K^+ (5.6×10^{-2} M) in a volume of 0.05 ml was given into the rat adrenal medulla before the treatment with SKF81297 and R-(-)-TNPA evoked the CA secretion of 172 ± 17 ng for 4 min. In the present work, high K^+ (5.6×10^{-2} M)-stimulated CA secretion after the pretreatment with SKF81297 (3×10^{-5} M) was inhibited to 40% of the corresponding control secretion (100 %) from 5 glands, as shown in Fig. 3. Also, R-(-)-TNPA (3×10^{-5} M) given for 60 min reduced high potassium-evoked CA secretory response to 60% of the corresponding control responses (100%), although they were not affected for 0-34 min period (Fig. 3).

Effects of SKF81297 and R-(-)-TNPA on DMPP-evoked CA secretion from the perfused rat adrenal glands

When perfused through the rat adrenal gland, DMPP (10^{-4} M), which is a selective neuronal nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (323±47 ng for 0~8 min). As shown in 4, DMPP-stimulated CA secretion following the loading with SKF81297 (3×10^{-5} M) for 60 min was relatively time-dependently inhibited to 50% of the control secretion (100%) from 5 adrenal glands. In the presence of R-(-)-TNPA (10^{-6} M), a selective dopaminergic D2 receptor agonist, for 60 min, DMPP-evoked

CA secretory response was also inhibited to 60% of the control release, although they were not affected for the first period (0-8 min), as shown in Fig. 4.

Effects of SKF81297 and R-(-)-TNPA on McN-A-343-evoked CA secretion from the perfused rat adrenal glands

In awaked rats, McN-A-343 induced hypertension and tachycardia, which are antagonized by propranolol (Martin, 1996). Adrenal demedullation had no effect on the tachycardia, whereas treatment with guanethidine suppressed both tachycardia and hypertension (Martin, 1996). It has been shown that muscarinic stimulation generates a depolarizing signal, which triggers the firing of action potentials, resulting in the increased CA release in the rat chromaffin cells (Akaike et al, 1990) and the perfused rat adrenal gland (Lim and Hwang, 1991). Therefore, it was of interest to examine the effects of SKF81297 and R-(-)-TNPA on CA secretion evoked by McN-A-343, which is a selective muscarinic M_1-receptor agonist (Hammer and Giachetti, 1982), in the isolated perfused rat adrenal glands.

As illustrated in Fig. 5, McN-A-343 (10^{-4} M), perfused into an adrenal vein for 4 min before the treatment with SKF81297 and R-(-)-TNPA caused an increased CA secretion by 157±15 ng (0-4 min). However, in the presence of SKF81297 (3 x 10^{-5} M), the CA secretory response evoked by McN-A-343 was diminished by 50% of the control release (100%) in a relative time-dependent fashion. Also, in the presence of R-(-)-TNPA (3 x 10^{-5} M) for 60 min, the CA secretion evoked by McN-A-343 was inhibited by 60% of the corresponding control release (100%), although they were not affected for 0-34 min period (Fig. 5).

Effects of SKF81297 and R-(-)-TNPA on Bay-K-8644-evoked CA secretion from the perfused rat adrenal glands

It has been found that Bay-K-8644 is a selective L-type calcium channel activator, which causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm et al., 1982; Wada et al., 1985) and enhances basal Ca^{2+} uptake (Garcia et al., 1984) and the CA release (Lim et al., 1992). Therefore, it was of interest to determine the effects of SKF81297 and R-(-)-TNPA on the CA secretion evoked by Bay-K-8644 from the isolated perfused rat adrenal glands. Fig. 6 illustrates the inhibitory effects of SKF81297 and R-(-)-TNPA on Bay-K-8644-evoked CA secretory responses. In the absence of SKF81297, Bay-K-8644 (10^{-5} M) given into the perfusion stream for 4 min produced CA secretion of 160±16 ng (0-4 min). However, in the presence of SKF81297 (3 x 10^{-5} M), the CA secretion evoked by Bay-K-8644 was time-dependently inhibited by 50% of the corresponding control release, as shown in Fig. 6. Also, R-(-)-TNPA (3 x 10^{-5} M), given into the adrenal gland for 60 min, reduced the CA secretory responses evoked by Bay-K-8644 to 62% of the corresponding control responses (100%), although they were not affected for 0-34 min period (Fig. 6).

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Effects of SKF81297 and R-(-)-TNPA on cyclopiazonic acid-evoked CA secretion from the perfused rat adrenal glands

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 (Georger & Riley, 1989; Seidler et al., 1989). It has been

shown that the mobilization of Ca^{2+} from Ca^{2+} stores by pharmacological manipulation (e.g., inhibition of Ca^{2+}-ATPase in the stores by thapsigargin or cyclopiazonic acid) activates Ca^{2+} entry through store-operated Ca^{2+} channel (SOC) (Takemura et al., 1989; Berridge, 1995; Fasolato et al., 1994). It was excitable to test the effects of SKF81297 and R-(-)-TNPA on the CA secretion evoked by cyclopiazonic acid from the isolated perfused rat adrenal glands. As shown in Fig. 7, cyclopiazonic acid (10^{-5} M)-evoked CA secretion prior to the treatment with SKF81297 (3×10^{-5} M) was 154±16 ng for 0-4 min. However, in presence of SKF81297 (3×10^{-5} M), the cyclopiazonic acid (10^{-5} M)-evoked CA secretion was inhibited by 58% of the control response (100%). Also, R-(-)-TNPA (3×10^{-5} M), given in to the adrenal medulla, also inhibited time-dependently the CA secretion evoked by cyclopiazonic acid by 66% of the corresponding control, although they were not affected for 0-49 min period (Fig. 7).

Effects of R(+)-SCH23390 and S(-)-raclopride on CA secretion evoked by ACh, high K+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

From the experimental results as shown in Fig. 2~7, both SKF81297 and R-(-)-TNPA showed relatively time-dependent inhibition in the CA secretory responses evoked by cholinergic stimulation and membrane depolarization form the perfused rat adrenal glands. Generally, the prototypical dopamine D_1 receptor agonist, 6-chloro-7,8-dihydroxy-1-phenyl- 2,3,4,5-tetrahydro-1H-3-benzazepine (SKF81297), and the dopamine D_1 receptor antagonist, (*R*)(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (R(+)-SCH23390), have been

widely used to characterize the functional role of dopamine D_1 receptors in both in vitro as well as in vivo paradigms (O'Boyle et al., 1989; Gessa et al., 1991; Lewis et al., 1998), Therefore, it was likely of interest to examine effects of R(+)-SCH23390 (a selective D_1 antagonist) and S(-)-raclopride (a selective D_2 antagonist), on CA secretion evoked by evoked by ACh, high K+, DMPP and McN-A-343 from the isolated perfused rat adrenal glands.

In perfused rat adrenal glands, both R(+)-SCH23390 and S(-)-raclopride themselves did not affect the CA secretory responses (data not shown). Therefore, in the subsequent experiments, the time-course effects of R(+)-SCH23390 and S(-)-raclopride on the CA seretory responses evoked by ACh, high K^+, DMPP and McN-A-343 were examined. In the present experiment, ACh (5.32 x 10^{-3} M)-evoked CA release prior to the perfusion with R(+)-SCH23390 or S(-)-raclopride was 362±19 ng (0-4 min). However, in the presence of R(+)-SCH23390 (3 x 10^{-6} M) and S(-)-raclopride (3 x 10^{-6} M) for 60 min, it was significantly increased by 118% (n=6) and 114% (n=5) of the control release only at first 0~4 min, respectively, but it was never altered at 15~64 min in comparison with the corresponding control release (Fig. 8). High potassium (56 mM KCl), a direct membrane-depolarizing agent, stimulates CA secretion (173±15 ng, 0-4 min). In the present work, high K^+ (5.6 x 10^{-2} M)-evoked CA release in the presence of R(+)-SCH23390 (3 x 10^{-6} M) and S(-)-raclopride (3 x 10^{-6} M)) for 60 min was greatly enhanced by 128% (n=5) and 131% (n=5) of the control release only at first 0-4 min, respectively, in comparison to the corresponding control secretion without alteration only at 15~64 min period, as shown in Fig. 9. DMPP (10^{-4} M), a selective nicotinic receptor agonist in autonomic sympathetic ganglia, when perfused through the rat adrenal gland,

evoked a sharp increase in CA secretion (397±17 ng, 0-8 min). As shown in Fig. 10, DMPP (10^{-4} M)-stimulated CA secretion following the loading with R(+)-SCH23390 (3 x 10^{-6} M) and S(-)-raclopride (3 x 10^{-6} M) was potentiated by 116% (n=5) and 113% (n=8) of the corresponding control secretion, respectively, which was also the peak release only at first 0-8 min without any alteration at 20-68 min period. As illustrated in Fig. 11, McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-receptor agonist (Hammer and Giachetti, 1982), perfused into an adrenal vein for 4 min caused an increased the CA secretion to 179±13 ng (0-4 min). However, in the presence of R(+)-SCH23390 (3 x 10^{-6} M) and S(-)-raclopride (3 x 10^{-6} M), McN-A-343-evoked CA secretion was significantly increased by 120% (n=5) and 131% (n=5) of the corresponding control release only at first 0-4 min, respectively, although there was no change at 15-64 min period.

Bay-K-8644 (10^{-5} M)-stimulated CA secretion in the presence of R(+)-SCH23390 (3 x 10^{-6} M) and S(-)-raclopride (3 x 10^{-6} M) was greatly enhanced to 139% (n=5) and 127% (n=8) of the corresponding control release (171±13 ng for 0-4 min) only at first 0-4 min, respectively, without any alteration at 15-64 min period, as shown in Fig. 12.

As depicted in Fig. 13, in the presence of R(+)-SCH23390 (3 x 10^{-6} M) and S(-)-raclopride (3 x 10^{-6} M), cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also potentiated to 125% (n=5) and 128% (n=5) of the corresponding control release (171±13 ng for 0-4 min) only at first 0-4 min, respectively, which was not changed at 15-64 min period.

IV. DISCUSSION

These experimental results obtained here demonstrate that both SKF81297 and R-(-)-TNPA inhibit the CA secretory responses evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization from the perfused rat adrenal medulla, but both R(+)-SCH23390 and S(-)-raclopride enhance the CA secretion by them. It seems that these inhibitory effects of SKF81297 and R-(-)-TNPA may be mediated by stimulation of dopaminergic D_1 and D_2 receptors located on the rat adrenomedullary chromaffin cells, respectively, while the facilitatory effects of R(+)-SCH23390 and S(-)-raclopride are due to the blockade of dopaminergic D_1 and D_2 receptors, respectively, which are relevant to extra- and intracellular calcium mobilization. Therefore, it is thought that the presence of both dopaminergic D_1 and D_2 receptors may be involved in regulation of the CA release in the rat adrenal medulla.

Generally, dopamine receptors (D_1–D_5) can be grouped into two receptor families. Dopamine D_2-like receptors include the dopamine D_2, D_3 and D_4 subtypes, whereas dopamine D_1-like receptors include the dopamine D_1 and D_5 subtypes (Neve and Neve, 1997). Dopamine D_1 receptors couple to the G_s protein family ($G\alpha_s$ and $G\alpha_{olf}$ isoforms) in recombinant cell systems (Dearry et al., 1990; Zhou et al., 1990) as well as in tissue (Zhuang et al., 2000; Corvol et al., 2001; Jin et al., 2001). The efficacy of ligands at dopamine D_1 receptors has generally been measured by determination of adenylyl cyclase activity (O'Boyle et al., 1989; Lewis et al., 1998; Cai et al., 1999). These receptors can be also

distinguished from each other by selective binding of various dopamine agonists and antagonists, sensitivity to toxins, and signal transduction pathways; D_1 dopamine receptors activate adenylyl cyclase, whereas D_2 receptors either inhibit adenylyl cyclase or have no effect (Kebabian et al., 1986; Vallar & Meldolesi, 1989).

Firstly, in support of the present results showing the inhibitory effect of SKF81297 on the CA release in the perfused rat adrenal medulla, previous data of Artalejo and his co-workers (1990) showed specific binding of the rhodamine conjugate of the D_1 antagonist SCH-23390 to almost all of the cells in chromaffin cell cultured. Because SCH-23390 will bind D_5 receptors as well as D_1 receptors, it is possible, given the results of RNA analysis by Dahmer and Senogles (1996), that D_5-receptors were labeled on the cells. These observations suggested that D_5 receptors on the cells are responsible for inhibition of secretion by D_1-selective agonists. However, either these D_5 receptors appeared to be unlinked to adenylyl cyclase, or the stimulation of adenylyl cyclase is so weak that it is undetectable in the assay (Dahmer and Senogles, 1996). There are reports suggesting that there are R(+)-SCH23390 binding sites that are not linked to adenylyl cyclase but may represent another D_1-like receptor (Andersen et al., 1990; Schoors et al., 1991), although no such receptor has yet been identified by cloning. These reports could be supported by the result of Dahmer and Senogles (1996) that D1-selective agonists inhibit secretagogue-stimulated Na^+ uptake into bovine adrenal chromaffin cells in a cyclic AMP-independent manner. However, Albillos and his colleagues (1992) have reached two conclusions: First, the cat adrenal medulla chromaffin cell possesses a dopamine D_1-receptor that seems to be coupled to an adenylyl cyclase. Second, this receptor regulates the muscarinic-mediated

catecholamine release response through a negative feed-back loop which uses cyclic AMP as a second messenger. In addition, D_1-like receptors have been reported to inhibit secretion (Schoors et aI., 1991).

These previous results are consistent with those obtained from the present study. In the present work, the pretreatment of R(+)-SCH23390, a selective dopaminergic D1-receptoe antagonist, relatively enhanced the CA secretory responses evoked by ACh, high K^+, DMPP and McN-A-343. This finding confirms that SKF81297 inhibits CA secretory responses evoked by cholinergic stimulation as well as membrane depolarization through activation of inhibitory dopaminergic D_1-receptors on adrenal medullary chromaffin cells of the rat. Furthermore, it has been underscored by the finding that bilateral infusion of the D_1 receptor agonist, SKF81297, into the rat prefrontal cortex (PFC) produced a dose-related impairment in spatial working memory that was reversed by D_1 antagonist pretreatment (Zahrt et al., 1996). In terms of these findings, it is plausible that dopaminergic D_1 receptors exist on the rat adrenomedullary chromaffin cells. It has also been reported that, in sinoaortic denervated dogs (i.e. animals deprived from baroreflex pathways), the fenoldopam-induced decrease in arterial blood pressure was more important than in normal dogs (Damase-Michel et al., 1995). Heart rate was unchanged. In these animals, dopaminergic D_1 stimulation induced a decrease in sympathetic tone, as shown by the significant fall in plasma noradrenaline levels. These "in vivo" data clearly demonstrate the inhibitory role of ganglionic D_1 receptors.

Secondly, in support of this idea showing the inhibitory effect of R-(-)TNPA on the CA release, it has been shown that the levels of NE in aqueous humor of the rabbit were reduced by 38% and 79% at 1 and 2 hr, respectively, following topical

application of R-(-)TNPA (Chu et al., 1999). Following pretreatment with raclopride, a D_2 receptor antagonist, and a subsequent challenge with R-(-)TNPA, the depression of intraocular pressure and levels of NE induced by R-(-)TNPA (2 hr) were antagonized. Thus, it is concluded that immunohistochemical identification of D_2 receptors in the ciliary body of the rabbit associated with the suppression of aqueous NE levels by topical application of the D_2 receptor agonist, R-(-)TNPA, provide strong evidence of prejunctional (neuronal) site of action of R-(-)TNPA (Chu et al., 1999). Antagonism of R-(-)TNPA-induced ocular hypotension by raclopride coupled with the immunohisto-chemical and NE data suggest that D_2 dopamine receptors are located on postganglionic sympathetic neurons in the ciliary body. Based on these findings, the present experimental results that R-(-)TNPA inhibited the CA secretion evoked by cholinergic stimulation as well as by membrane depolarization, while S-(-) raclopride, a selective dopaminergic D_2 antagoist, enhanced the CA secretion by them strongly suggest that R-(-)TNPA can suppress the CA secretion through the the activation of the the inhibitory dopaminergic D_2 receptors located on the rat adrenomedullary chromaffin cells. It has also been found that the presence of D_2 dopamine receptors on adrenal chromaffin cells is demonstrated in several studies by radioligand binding methods (Gonzalez et al., 1986; Lyon et al., 1987; Quick et al., 1987). These dopamine receptors located on chromaffin cells appear to function as an inhibitory modulator of adrenal CA secretion as shown in the results obtained in the cultured bovine adrenal chromaffin cells (Bigornia et al., 1988; 1990) and in some studies with the perfused cat adrenal glands (Artalejo et al., 1985; Gonzalez et al., 1986; Montastruc et al., 1989). Moreover, it has also been reported that the bovine adrenal glands contain dopaminergic receptors

that modulate CA secretion evoked by stimulation of the nicotinic cholinergic receptors through activation of the inhibitory D_2 type receptors (Gonzalez et al., 1986). Subcutaneous injection of apomorphine in normotensive rats has been found to produce a dose-dependent decrease in CA content of the adrenal gland via the activation of dopaminergic D_2-receptor probably located on splanchnic nerve endings (Montastruc et al., 1989). The investigational data obtained in the bovine adrenal chromaffin cells could support that dopaminergic D_2-receptors appear to function as inhibitory modulators of adrenal CA secretion (Bigornia et al., 1988; 1990). Furthermore, these inhibitory effects of apomorphine or dopamine on nicotine-evoked CA secretion are antagonized or reversed by the pretreatment with dopaminergic D_2 antagonists, domperidore, sulpiride, haloperidol and metoclopramide (Collet & Story, 1982a; 1982b; Artalejo et al., 1985; Bigornia et al., 1988; 1990; Montiel et al., 1986; Montastruc et al., 1989; Lim et al., 1994). These previous results are consistent with those obtained from the present study. In terms of these results, in the present work the pretreatment of S-(-) raclopride, a selective dopaminergic D2 antagonist, enhanced the CA secretory process evoked by ACh, high K^+, DMPP and McN-A-343. This fact confirms that R-(-)TNPA inhibits CA secretory responses evoked by nicotinic stimulation as well as membrane depolarization through the activation of inhibitory dopaminergic D_2 -receptors on adrenal medullary chromaffin cells of the rat. Collet and Story (1982a) have found that dopamine inhibited the electrically evoked release of [^{3}H] NE from isolated perfused rabbit adrenal glands. This inhibition could be reversed completely by the dopamine D_2 selective antagonist, metoclopramide. In the previous experiments, it has been known that metoclopramide evokes CA secretion in the perfused rat adrenal gland (Lim et al.,

1989). Moreover, It has also been demonstrated that apomorphine causes a dose-dependent inhibition of CA secretion by cholinergic receptor stimulation and also by membaren depolarization from the isolated perfused rat adrenal gland (Lim et al., 1994).

Thus, the results of the present work that R-(-)TNPA causes the inhibition of CA secretory responses evoked by ACh, DMPP, McN-A-343 and excess K^+ through D_2 dopaminergic activation can be supported by several previous studies (Gonzalez et al., 1986; Lyon et al., 1987; Quick et al., 1987; Damase-Michel et al., 1999), although the activation of D_1 receptors by SKF81297 also inhibits these secretagoues-evoked CA release. Consistently with the present results, dopaminergic inhibitory effects in other systems were found to be mediated specifically by the D_2-receptor subtype (Memo et al., 1985; de Vliefer et al., 1985; Cooper et al., 1986; Malgaroli et al., 1987). Moreover, Bigornia and his colleague (1990) have demonstrated that, in the same preparation of adrenomedullary samples where significant numbers of D_2 receptors are found there is no statistical significant specific binding of the D_1 receptor ligand, [^{3}H] SCH 23390. Dopaminergic inhibition of CA secretion from adrenal medulla of conscious male beagle dogs was found to be mediated by D_2-like but not D_1-like dopaminergic receptors (Damase-Michel et al., 1999).

In the present investigation, the finding that SKF81297 and R-(-)TNPA time-dependently inhibited the CA secretory responses evoked by high K^+ as well as by Bay-K-8644, which specifically activates an L-type, voltage-sensitive calcium channel, suggests that SKF81297 and R-(-)TNPA-induced inhibitory effect on CA release is due to the blockade of the voltage-sensitive calcium channels. The results of the present work also illustrate that SKF81297 and

R-(-)TNPA produce the inhibitory modulation of adrenal CA secretion at least partly by inhibition of calcium channel currents through stimulation of both dopaminergic D_1 and D_2 receptors. In support of this idea, there are now sizeable literatures demonstrating that plays a key role of calcium influx through voltage-sensitive Ca^{2+} channels in a physiological pathway for activation of adrenal CA secretion (Douglas, 1975; Holz et al., 1982; Kilpatrick et al., 1982; Cena et al., 1983; Knight & Kestevan, 1983; Kao & Schneider, 1986). Bigornia and his coworkers (1988) showed that apomorphine caused a dose-dependent inhibition of $^{45}Ca^{2+}$ uptake stimulated by nicotine or membrane depolarization with an elevation K^+ level as well as Bay-K-8644, a calcium channel activator. This inhibition of $^{45}Ca^{2+}$ uptake stimulated by these agents was reversed by a series of specific dopaminergic receptor antagonists. These effects are fully in agreement with the present experimental data. In view of the results obtained from the present experiment, it is felt that the voltage-sensitive calcium channel located on chromaffin cell membrane of the rat adrenal medulla could be the target site for both dopaminergic D_1 and D_2 receptor-mediated inhibition of CA secretion.

In the present study, SKF81297 and R-(-)TNPA also inhibited the cyclopiazonic acid-evoked CA secretory responses. Cyclopiazonic acid is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Siedler et al., 1989). Therefore, it is thought that the inhibitory effect of SKF81297 and R-(-)TNPA on 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 result indicates that the activation of dopaminergic D_1 and D_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, SKF81297 and R-(-)TNPA produced time-dependently inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests new other concept that SKF81297 and R-(-)TNPA 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; Challis et al., 1991). However, in the present study, it is uncertain whether the inhibitory effect of the SKF81297 and R-(-)TNPA 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 the activation of dopaminergic D1- and D2-receptors.

In contrast with the present experimental results, Huettl and his colleagues (1991) concluded that pergolide and apomorphine act in a nonreceptor-mediated manner to inhibit CA release from bovine chromaffin cells and that functional dopaminergic D_2 receptors of the classical type do not exist on isolated bovine chromaffin cells. Because the inhibitory effect of the selective dopaminergic D_2 agonists pergolide as well as apomorphine on CA release from the chromaffin

cells was neither reversed nor antagonized by the selective dopaminergic D_2 receptor antagonists such as haloperidol, domperidone, metoclopramide, fluphenazine, flugintixol and sulpiride (Huettl et al., 1991). It has also been shown that stimulation of dopaminergic D_1-receptors activates the facilitation of Ca^{2+} currents in the absence of pre-depolarizations or repetitive activity from bovine chromaffin cells, and that activation by D_1 agonists is mediated by cAMP and protein kinase A (Artalejo et al., 1990). This recruitment of facilitation of Ca^{2+} channels by dopamine may form the basis of a positive feedback loop mechanism that augments CA secretion. Facilitation of exocytosis is mediated by interaction of a released substance with a D1 dopamine receptor (Artalejo et al., 1991), the conditions that showed facilitation were repeated in the presence of SCH-23390, a D1 antagonist. While this agent blocked facilitated release in a dose-dependent manner, facilitated release was unaffected by the D2 antagonist, raclopride. The doses of SCH-23390 needed to inhibit facilitation are quite high. Two possibilities may contribute to this finding. First, the binding of SCH-23390 to sites on chromaffin cell is considerably weaker than to other tissues that have D1 receptor sites (Dahmer and Senogles, 2000). Because of this, this facilitation seems to be probably due to this receptor as a "D1-like receptor." Second, the assay used involves the competition between released epinephrine and the antagonist. Further evidence that a D1-like receptor is involved in facilitation was obtained with the D1 agonist, SKF-38393 (Villanueva and Wightman, 2007). This agent induced exocytotic facilitation in a 30 s paired pulse intervals, a paradigm that normally shows limited facilitation. In anesthetized dogs, both quinpirole and apomorphine, selective D_2 dopaminergic agonists, did fail to modify release of EP and NE from the adrenal medulla whatever the stimulation frequencies of the

sectioned splanchnic nerve. This fact indicates that peripheral dopaminergic D_2 receptors are not involved in the control of CA release from the adrenal medulla under *in vivo* conditions (Damase-Michel et al., 1990).

As shown in Fig. 14, conclusively, these results demonstrate that both SKF81297 and R-(-)TNPA greatly inhibit the release of CA from the isolated perfused rat adrenal gland evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization without affecting the basal release. It seems likely that the inhibitory effects of SKF81297 and R-(-)TNPA are mediated by the activation of D_1-like and D_2-like dopaminergic receptors located on the rat adrenomedullary chromaffin cells, respectively: this action is possibly associated with extra- and intracellular calcium mobilization. Therefore, it is thought that the presence of dopaminergic D_1 receptors may play an important role in regulation of the rat adrenomedullary CA secretion, in addition to well-known dopaminergic D_2 receptors.

V. SUMMARY

Dahmer and Senogles (1996) have observed that the D_1-selective agonists CI-APB and SKF-38393 inhibit DMPP-stimulated secretion of catecholamines (CA) in a concentration-dependent manner. However, in contrast to these findings, it has been reported that dopaminergic inhibition of CA secretion from adrenal medulla of conscious male beagle dogs is mediated by D_2-like but not D_1-like dopaminergic receptors (Damase-Michel et al., 1999), and the inhibitory D_2 dopaminergic effect of CA secretion from the bovine chromaffin cells is not to interact D_1 receptors as described previously (Bigornia et al., 1988; 1990). Therefore, the present study was designed to establish comparatively the inhibitory effects of D_1-like and D_2-like dopaminergic receptor agonists, SKF81297 and R(-)-TNPA on the CA release evoked by cholinergic stimulation and membrane depolarization from the isolated perfused model of the rat adrenal medulla.

SKF81297 (30 μM) and R-(-)-TNPA (30 μM) perfused into an adrenal vein for 60 min, respectively, produced greatly inhibition in the CA secretory responses evoked by ACh (5.32×10^{-3} M), DMPP (10^{-4} M), McN-A-343 (10^{-4} M), high K^+ (5.6×10^{-2} M), Bay-K-8644 (10 μM), and cyclopiazonic acid (10 μM), respectively. For the release of CA evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid, the following rank order of inhibitory potency was obtained: SKF81297>R-(-)-TNPA. However, R(+)-SCH23390, a selectve D_1-like dopaminergic receptor antagonist, and S(-)-raclopride, a selectve D_2-like dopaminergic receptor antagonist, enhanced the CA secretory responses evoked

by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid only for 0-4 min. The rank order for the enhancement of CA release evoked by high K^+, McN-A-343 and cyclopiazonic acid was R(+)-SCH23390> S(-)-raclopride. Also, the rank order for ACh, DMPP and Bay-K-8644 was S(-)-raclopride > R(+)-SCH23390. However, SKF81297, R(-)-TNPA, R(+)-SCH23390 and S(-)-raclopride themselves did not affect the spontaneous CA release from the arenal medulla.

Taken together, these results demonstrate that both SKF81297 and R-(-)-TNPA greatly inhibit the release of CA evoked by stimulation of cholinergic (both nicotinic and muscarinic) receptors and the membrane depolarization from the isolated perfused rat adrenal gland without affecting the basal release, respectively, but both R(+)-SCH23390 and S(-)-raclopride facilitate the CA release evoked by them. It seems likely that the inhibitory effects of SKF81297 and R-(-)-TNPA are mediated by the activation of D_1-like and D_2-like dopaminergic receptors located on the rat adrenomedullary chromaffin cells, respectively, whereas the facilitatory effects of R(+)-SCH23390 and S(-)-raclopride are mediated by the blockade of D_1-like and D_2-like dopaminergic receptors, respectively: this action is possibly associated with extra- and intracellular calcium mobilization. Therefore, it is thought that the presence of dopaminergic D_1 receptors may play an important role in regulation of the rat adrenomedullary CA secretion, in addition to well-known dopaminergic D_2 receptors.

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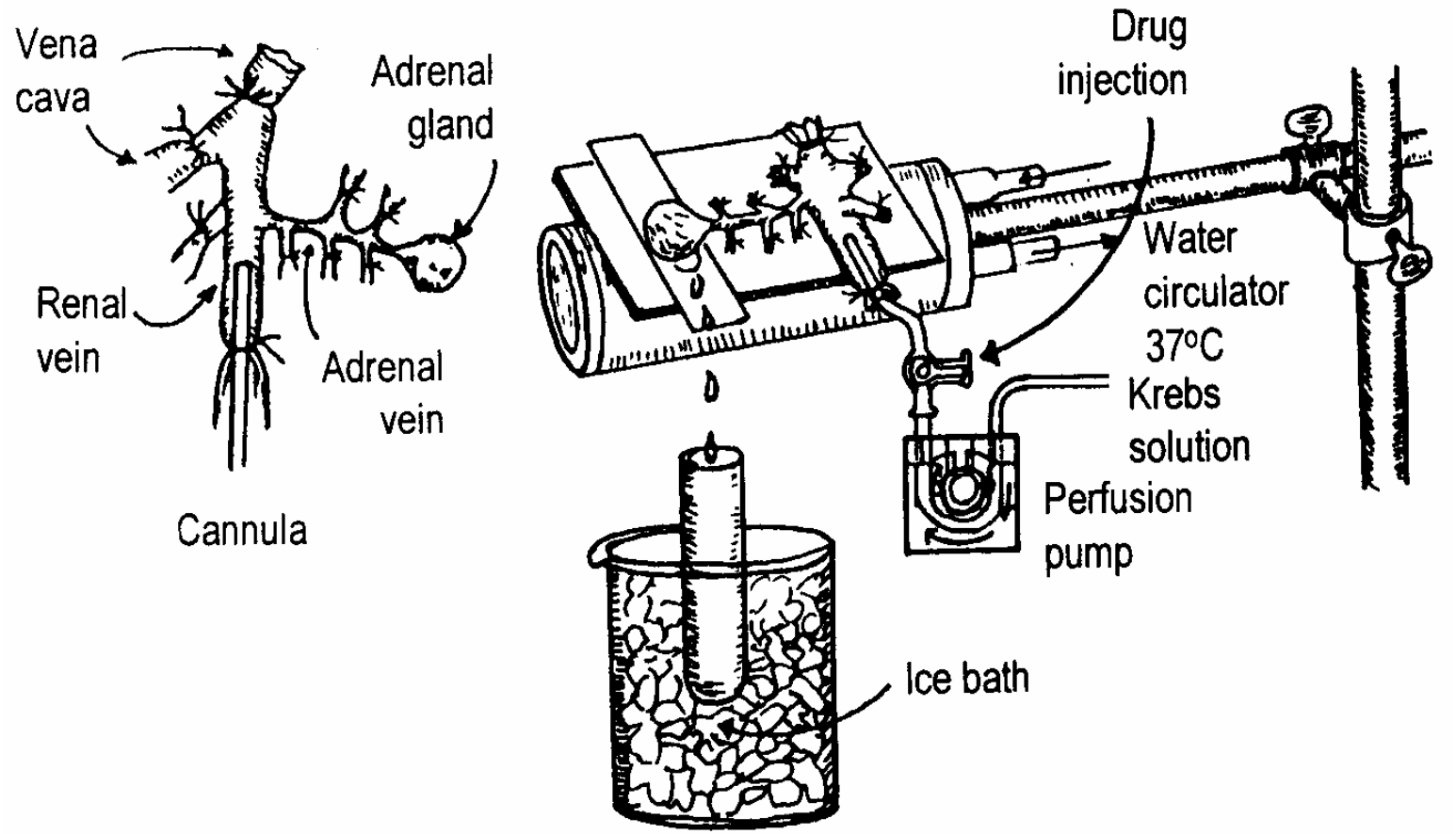

Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamines in the isolated perfused adrenal gland of the SHRs.

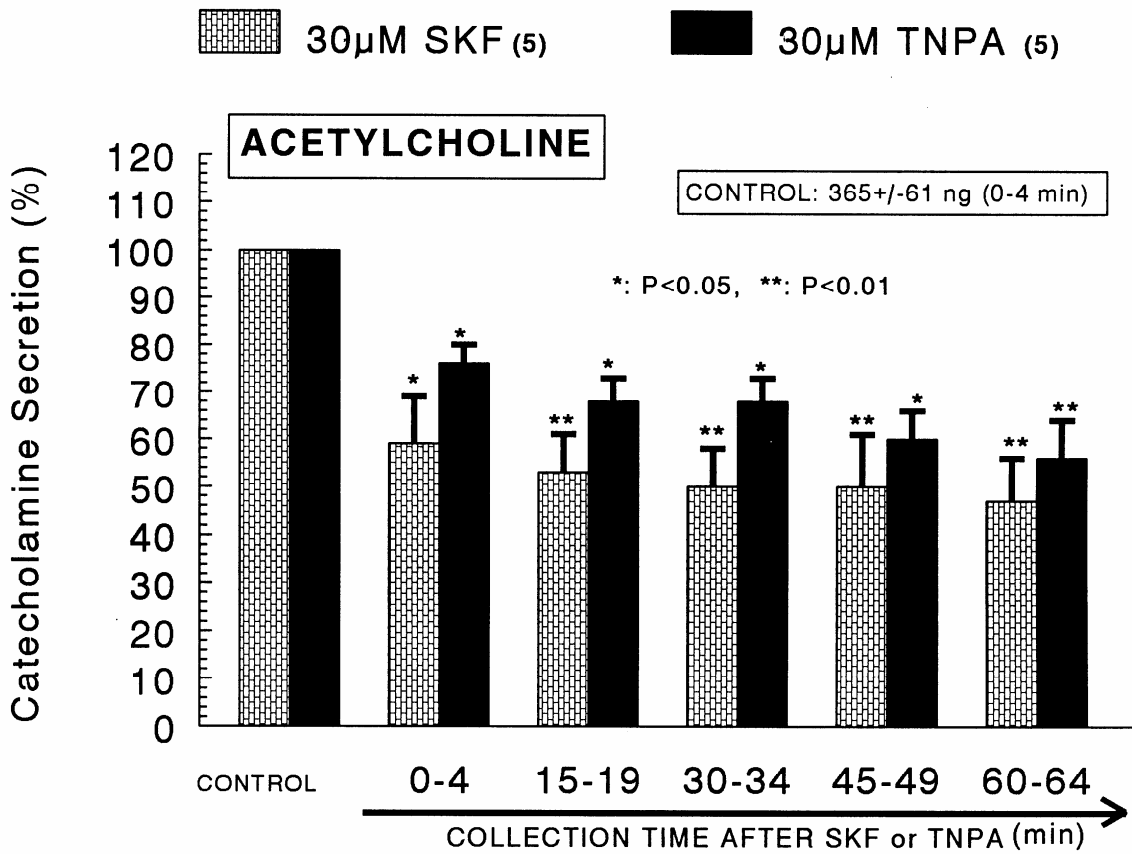

Fig. 2. Time course effect of SKF81297 (SKF) and R-(-)-TNPA (TNPA) on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. The CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively, 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). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control with the pretreated group with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively. ACh-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

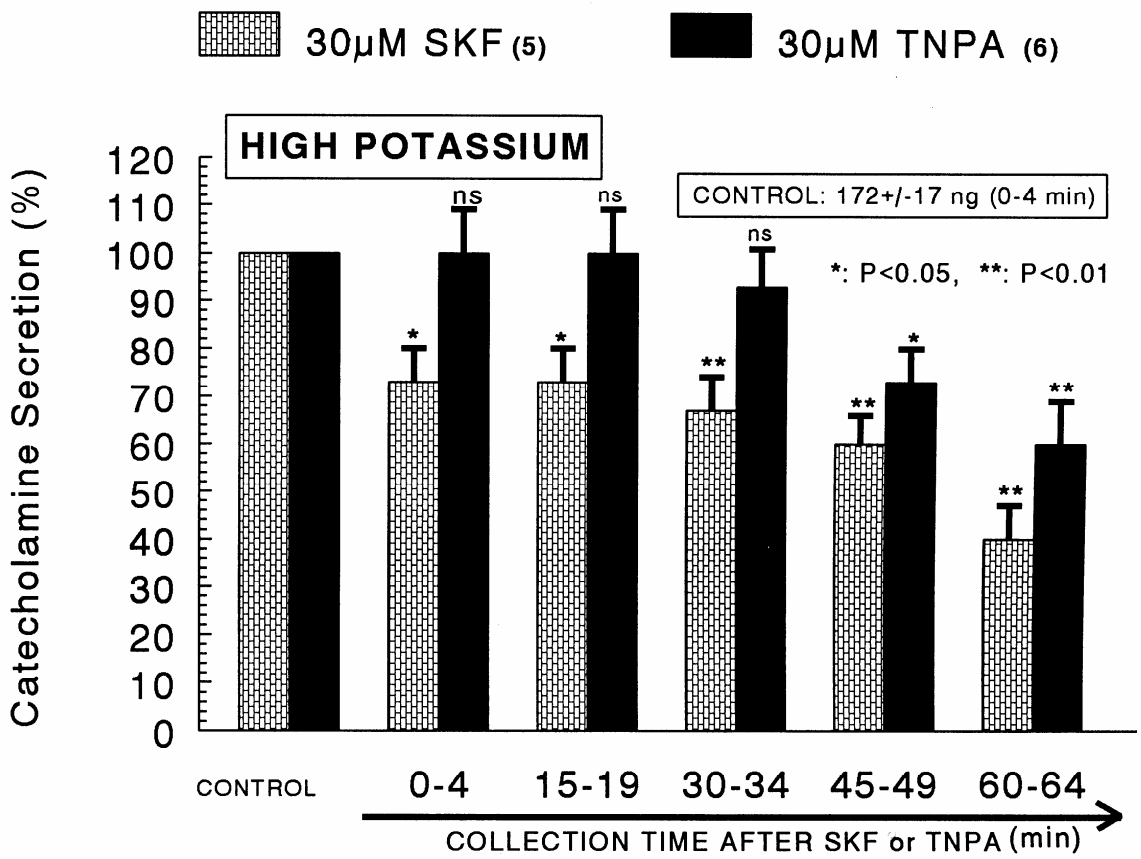

Fig. 3. Time course effect of SKF81297 (SKF) and R-(-)-TNPA (TNPA) on the secretory responses of catecholamines (CA) evoked by high potassium from the isolated perfused rat adrenal glands. The CA secretion by a single injection of high K^+ (5.6×10^{-2} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. High K^+-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$.

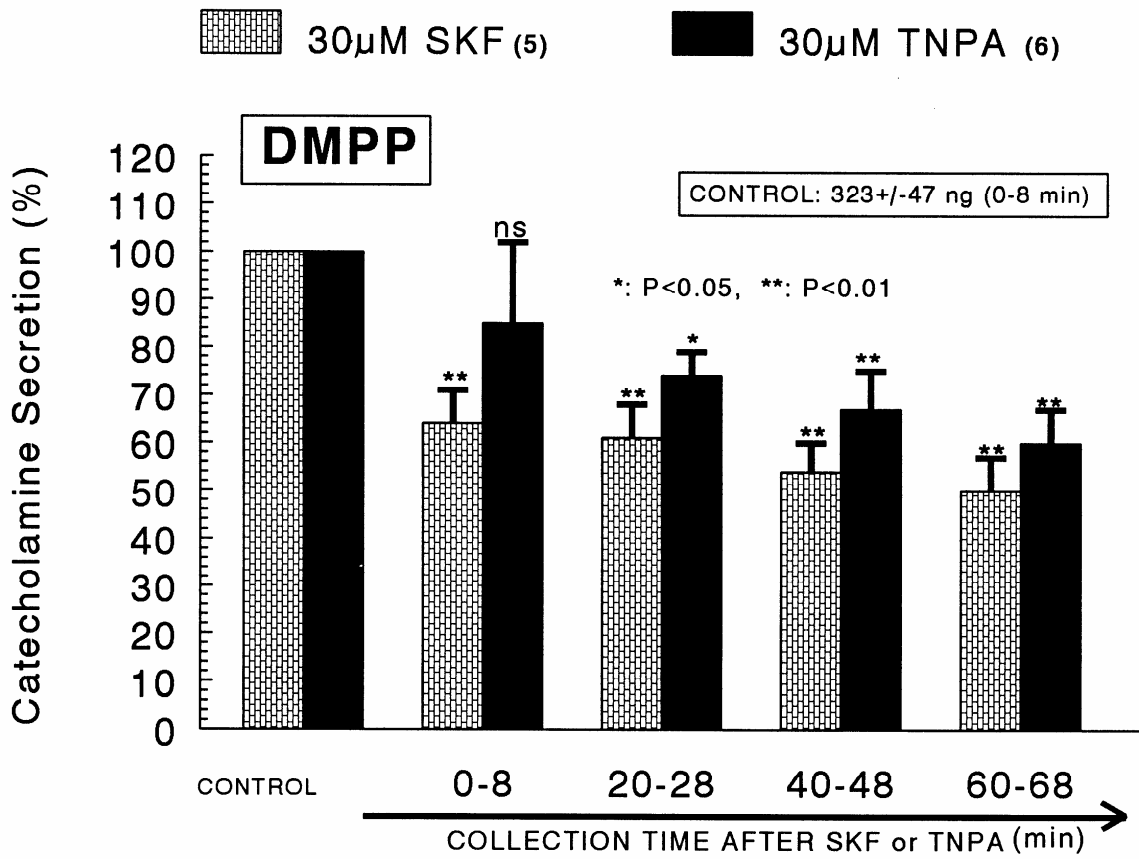

Fig. 4. Time course effect of SKF81297 (SKF) and R-(-)-TNPA (TNPA) on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. The CA secretion by infusion of DMPP (10^{-4} M) for 2 min was evoked at 20 min intervals after preloading with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. DMPP-induced perfusate was collected for 8 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

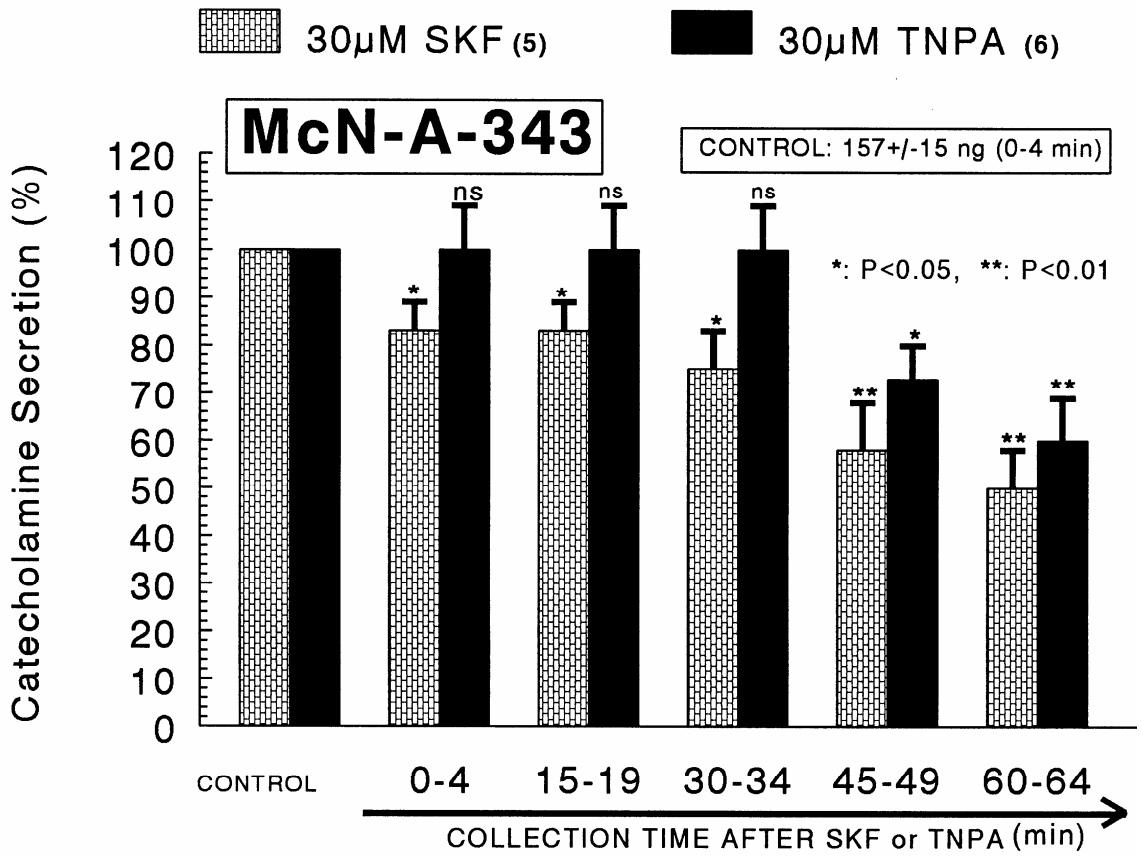

Fig. 5. Time course effect of SKF81297 (SKF) and R-(-)-TNPA (TNPA) on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. The CA secretion by infusion of McN-A-343 (10^{-4} M) for 4 min was evoked at 15 min intervals after preloading with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. McN-A-343-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

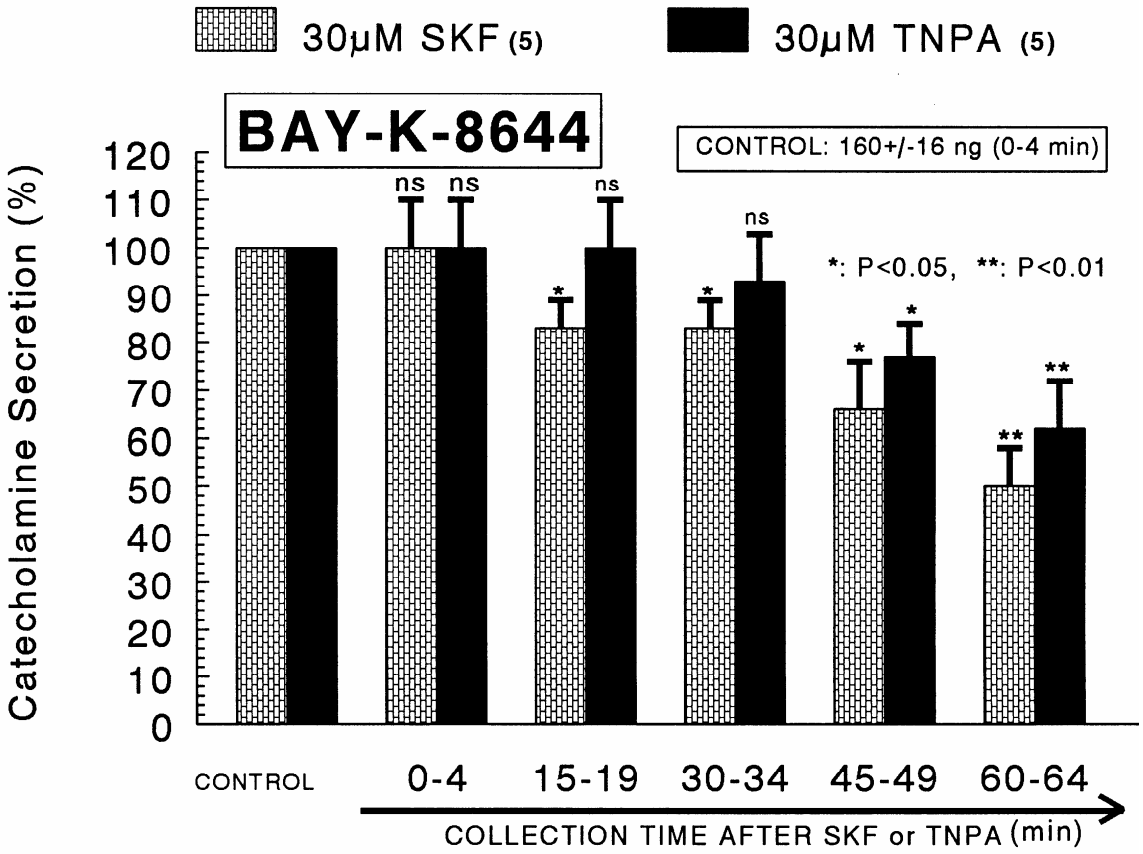

Fig. 6. Time course effect of SKF81297 (SKF) and R-(-)-TNPA (TNPA) on the secretory responses of catecholamines (CA) evoked by Bay-K-8644 from the isolated perfused rat adrenal glands. The CA secretion by infusion of Bay-K-8644 (10^{-5} M) for 4 min was evoked at 15 min intervals after preloading with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. Bay-K-8644-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

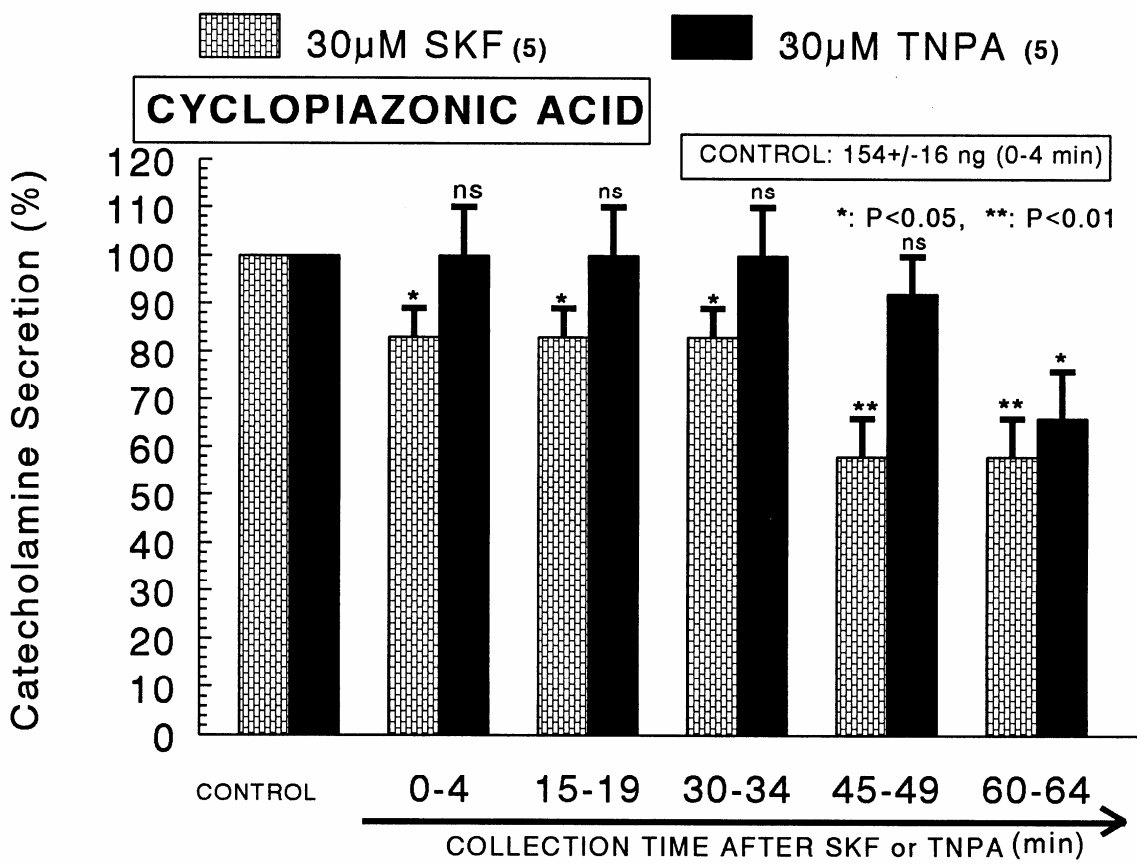

Fig. 7. Time course effect of SKF81297 (SKF) and R-(-)-TNPA (TNPA) on the secretory responses of catecholamines (CA) evoked by cyclopiazonic acid from the isolated perfused rat adrenal glands. The CA secretion by infusion of cyclopiazonic acid (10^{-5} M) for 4 min was evoked at 15 min intervals after preloading with SKF (30 μM) and TNPA (30 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. cyclopiazonic acid-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

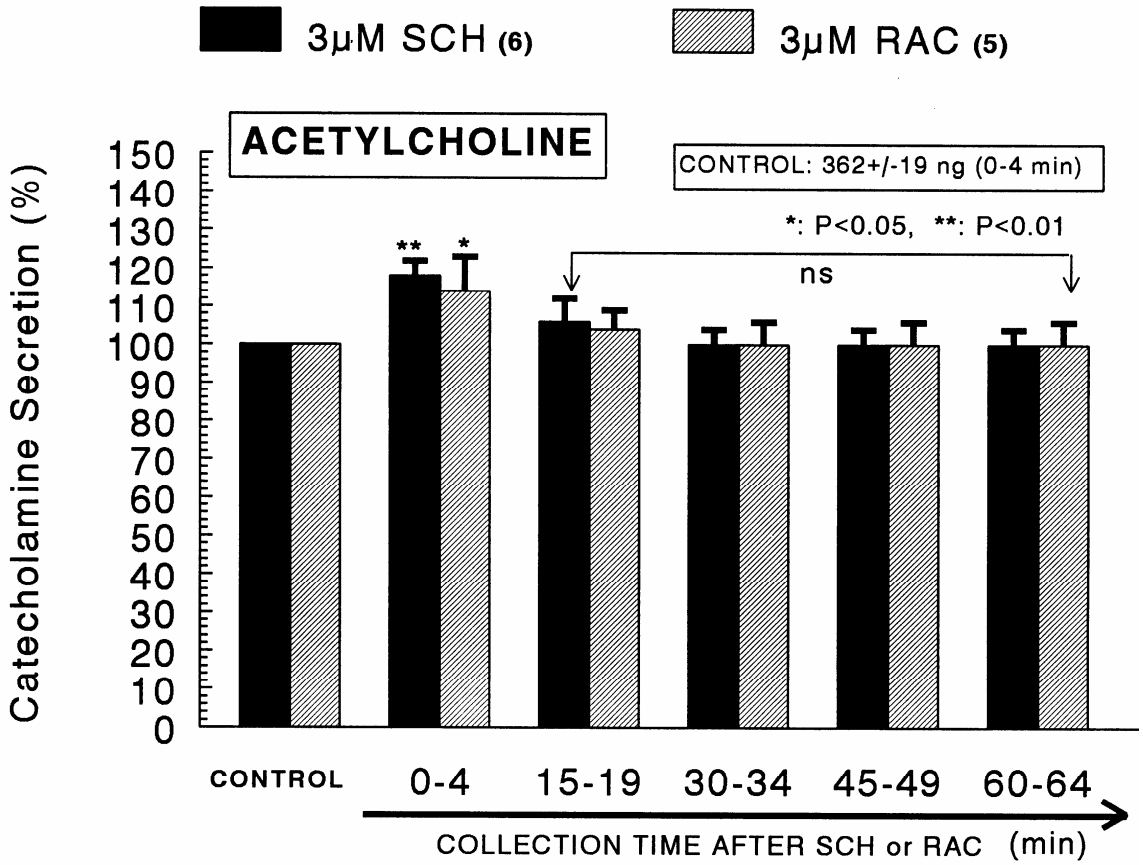

Fig. 8. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh) from the isolated perfused rat adrenal glands. The CA secretion by a single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with SCH (3 μM) and RAC (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. ACh-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

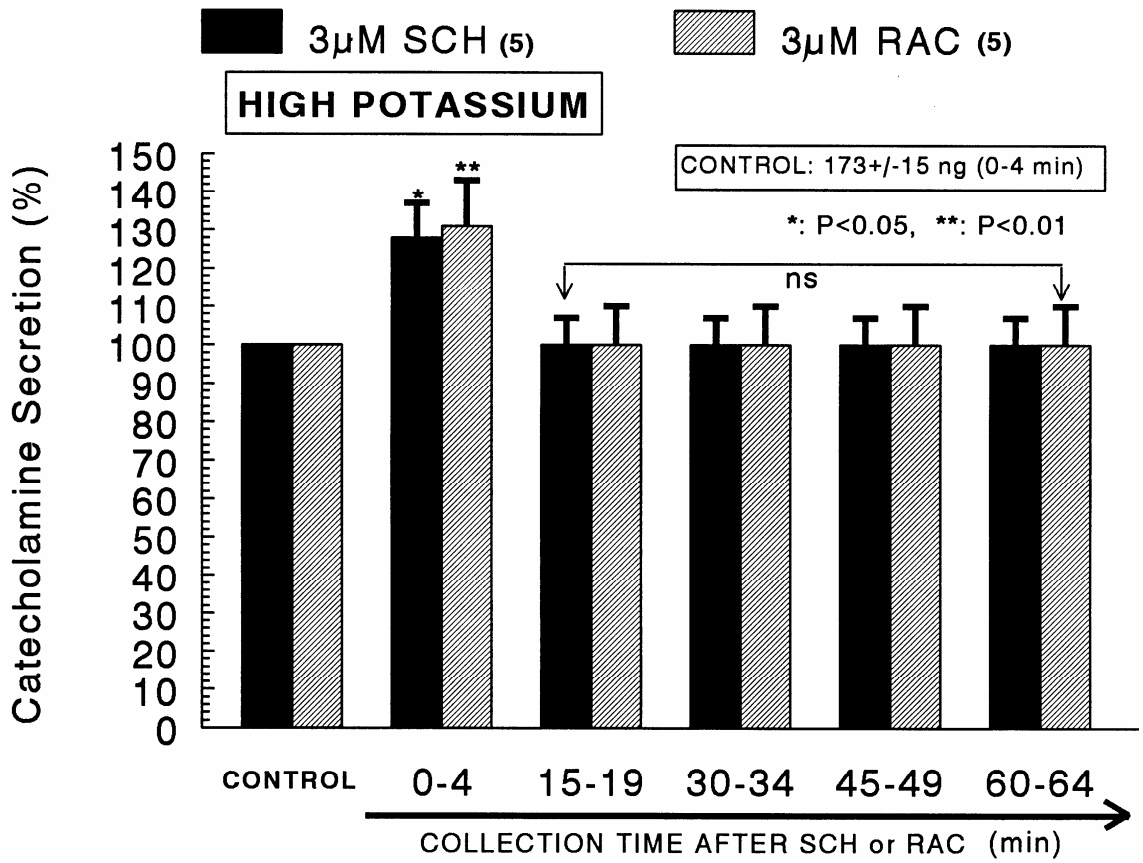

Fig. 9. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the secretory responses of catecholamines (CA) evoked by high potassium from the isolated perfused rat adrenal glands. The CA secretion by a single injection of high K^+ (5.6 x 10^{-2} M) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with SCH (3 μM) and RAC (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. High K^+-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

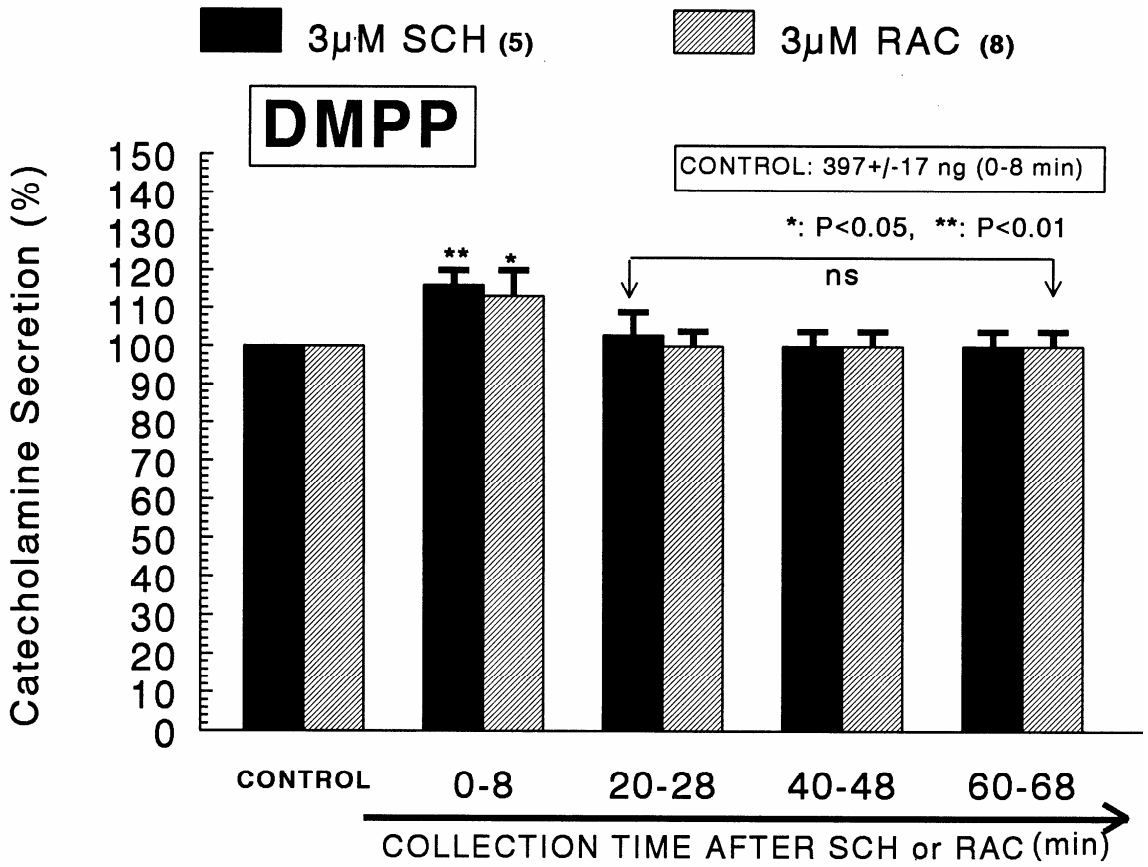

Fig. 10. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rat adrenal glands. The CA secretion by infusion of DMPP (10^{-4} M) for 2 min was evoked at 20 min intervals after preloading with SCH (3 μM) and RAC (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. DMPP-induced perfusate was collected for 8 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

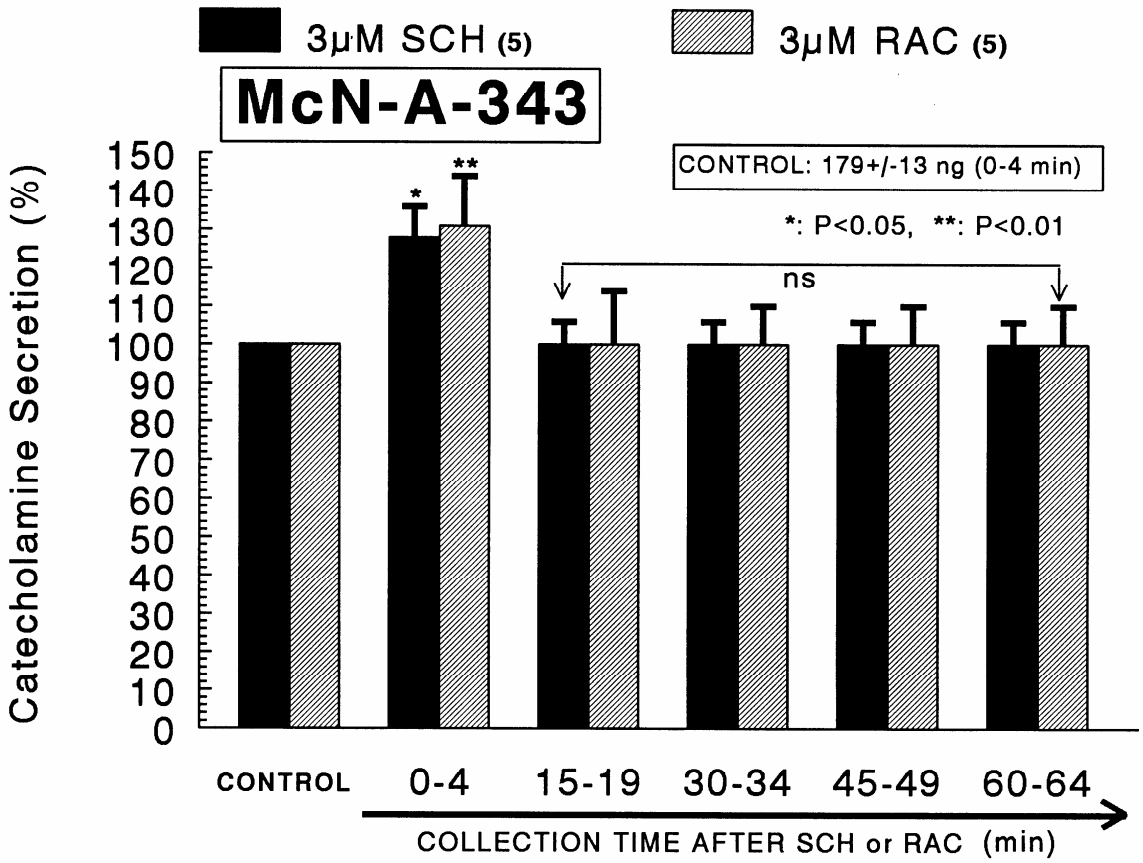

Fig. 11. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the secretory responses of catecholamines (CA) evoked by McN-A-343 from the isolated perfused rat adrenal glands. The CA secretion by infusion of McN-A-343 (10^{-4} M) for 4 min was evoked at 15 min intervals after preloading with SCH (3 μM) and RAC (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. McN-A-343-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

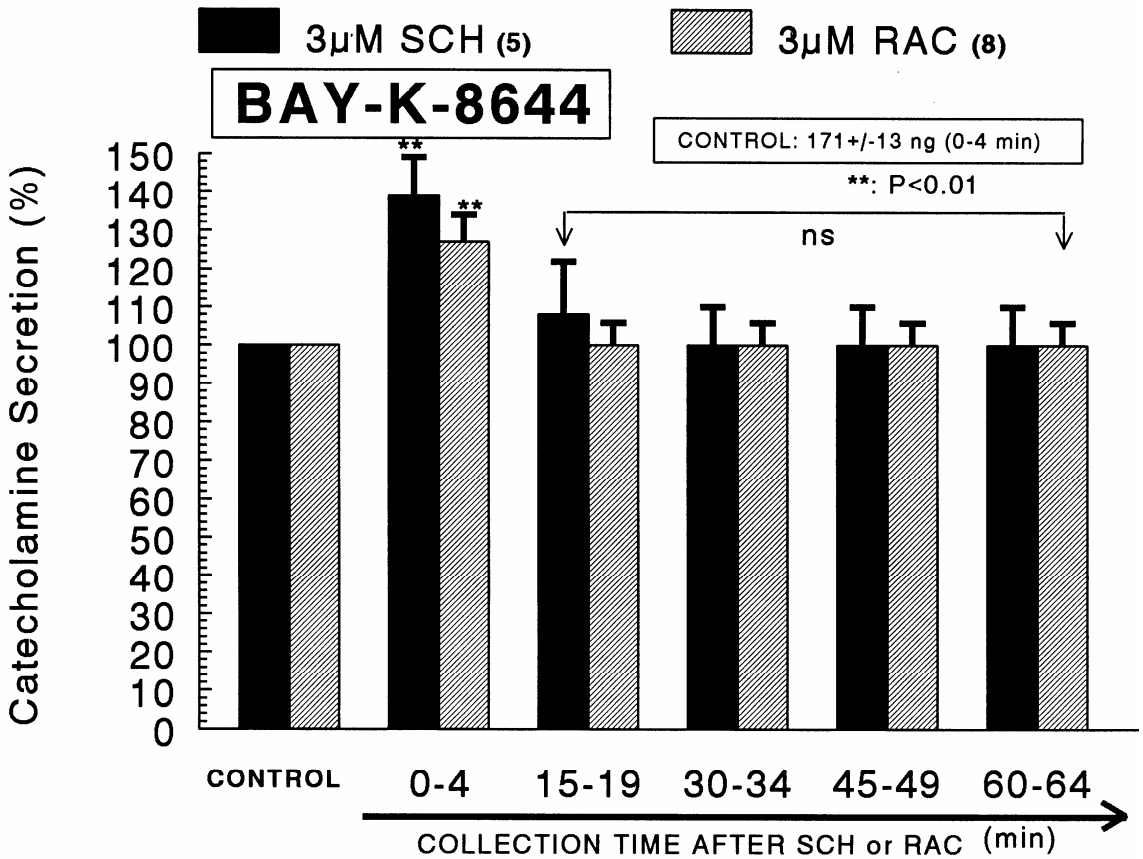

Fig. 12. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the secretory responses of catecholamines (CA) evoked by Bay-K-8644 from the isolated perfused rat adrenal glands. The CA secretion by infusion of Bay-K-8644 (10^{-5} M) for 4 min was evoked at 15 min intervals after preloading with SCH (3 μM) and RAC (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. Bay-K-8644 -induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

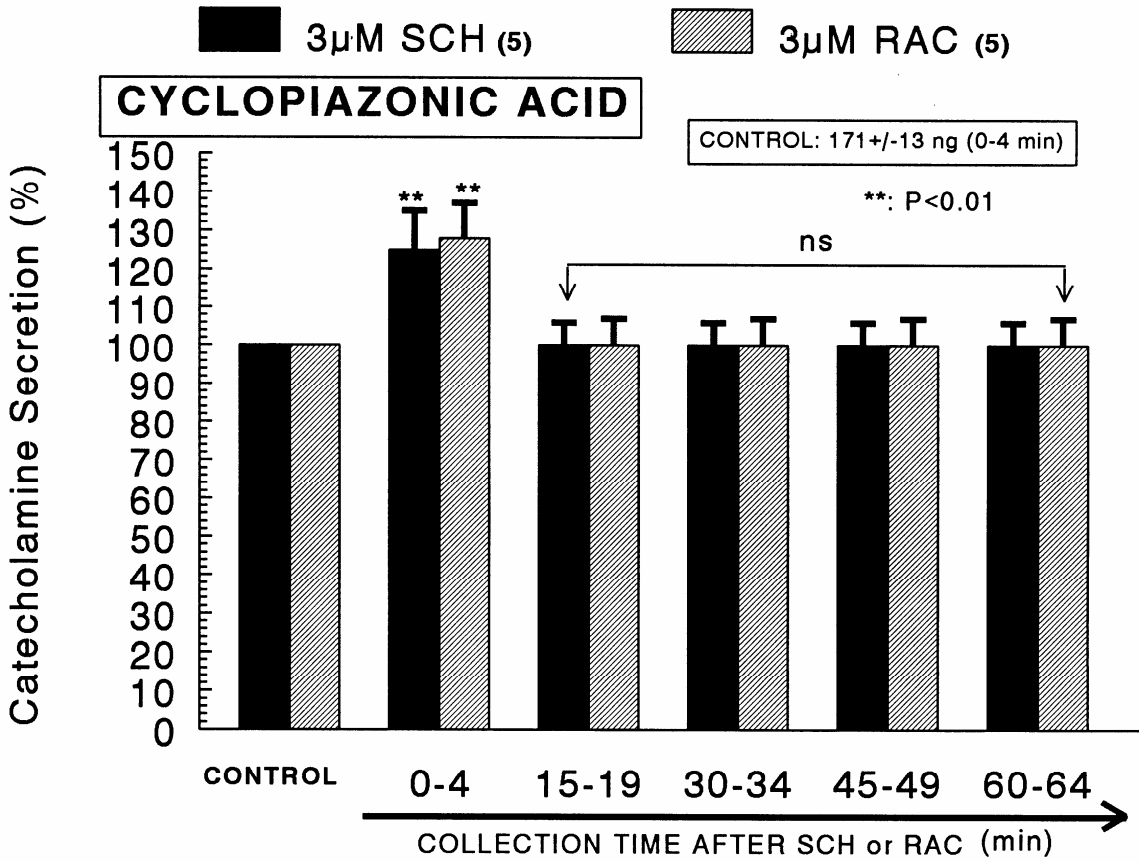

Fig. 13. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the secretory responses of catecholamines (CA) evoked by cyclopiazonic acid from the isolated perfused rat adrenal glands. The CA secretion by infusion of cyclopiazonic acid (10^{-5} M) for 4 min was evoked at 15 min intervals after preloading with SCH (3 μM) and RAC (3 μM) for 60 min, respectively, as indicated at an arrow mark. The other legends are the same as in Fig. 2. cyclopiazonic acid-induced perfusate was collected for 4 min. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

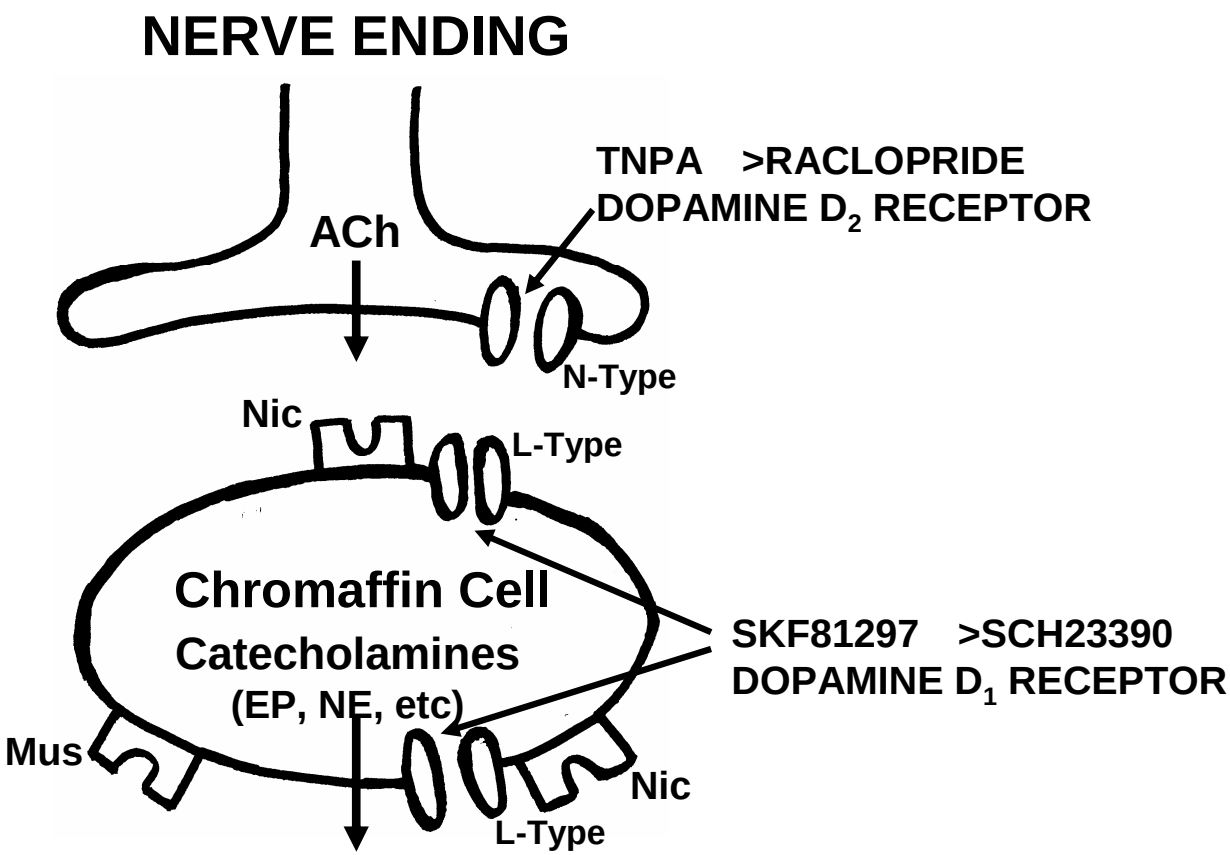

Fig. 14. Schematic diagram of possible action site of dopaminergic D_1 and D_2 receptor agonists at the cholinergic nerve ending-chromaffin cell synapse in the the rat adrenal gland. This diagram demonstrates possible localizations of voltage-dependent Ca^{2+} channels and cholinergic receptors mediating adrenal catecholamine secretion. Catecholamine-containing cells possess synaptic nicotinic receptors, extrasynaptic nicotinic and muscarinic receptors, and L-type voltage-dependent Ca^{2+} channels close to the synaptic and extrasynaptic nicotinic receptors. N-type voltage-dependent Ca^{2+} channenls are located on cholinergic nerve endings.