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

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The aim of 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 release of catecholamines (CA) 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, produced great 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. Taken together, these results demonstrate that both SKF81297 and R-(-)-TNPA inhibit the CA release 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. Based on these results, 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.

Key Words: SKF81297, R-(-)TNPA, R(+)-SCH23390, S(-)-raclopride, Catecholamine secretion, Adrenal meduula, Dopaminergic receptors

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 D_2-like (the D_2 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 at bovine chromaffin cells from the adrenal gland has been reported. 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 D_2 dopamine receptors are located on bovine chromaffin cells and their activation inhibited release (Bigornia et al., 1990). In chromaffin cells, inhibition of the CA secretion by dopamine agonists has been attributed to D_2 dopamine receptors based on the reversal of inhibition by D_2-selective antagonists such as

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ABBREVIATIONS: R-(-)-TNPA, R-(-)-2, 10, 11-trihydroxy-N-propyl-noraporphine hydrobromide; CA, catecholamines; SKF81297, 6-chloro-7, 8-dihydroxy-1-phenyl-2, 3, 4, 5-tetrahydro-1H-3-benzazepine; R(+)-SCH 23390, (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide, methyl-1,4-dihydro-2; BAY-K8644, 6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate; McN-A-343, 3-(m-cholro-phenyl-carbamoyl-oxy)-2-butynyltrimethyl ammonium chloride.

haIoperidol and butaclamol (Artalejo et aI., 1985; Bigornia et al., 1988). In addition, binding sites for tritiated antagonists selective for the D_2 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 D_2 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 D_2-like receptor agonist, quinpirole, had an inhibitory effect, while the dopamine D_2-like receptor antagonist, domperidone, had a stimulatory effect on norepinephine (NE) and epinephrine (EP) release (Foucart et al., 1988). Stimulation of NE and EP 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 D_2-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 epinephrine 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 D_2 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 D_1-like receptor (the D_1 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 D_1 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 D_1 and D_2 receptors exist on the rat adrenomedullary chromaffin cells. The second aim of the present study is to investigate whether the activation of both dopaminergic D_1 and D_2 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 D_2 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 D_2 receptors, and to establish the its mechanism of action.

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 upper 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 lower side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37±1°C.

Perfusion of adrenal gland

The adrenal glands were perfused by means of 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 DMPP (10^{-4} M) for 2 minutes and/or a single injection of ACh (5.32×10^{-3} M) and KCl (5.6×10^{-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-(-)-TNPA 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*-test. 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.

RESULTS

Effects of SKF81297 and R-(-)-TNPA on catecholamine (CA) secretion evoked by acetylcholine and high potassium 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 D_2 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. 1 (Upper). Also, in the presence of R-(-)-TNPA (3×10^{-5} M), a selective dopamine D_2 receptor agonist, for 60 min, ACh-evoked CA secretory response was inhibited to 56% of the control release (Fig. 1-Upper).

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. 1 (Lower). 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. 1-Lower).

Effects of SKF81297 and R-(-)-TNPA on the CA secretion evoked by DMPP and McN-A-343 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 2 (Upper), 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 D_2 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. 2 (Upper).

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. 2 (Lower), 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 (157±15 ng, 0～4 min). However, in the presence of SKF81297 (3×10^{-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 (3×10^{-5} M) for 60 min, the CA secretion evoked by McN-A-343 was inhibited by 60% of the corresponding control release

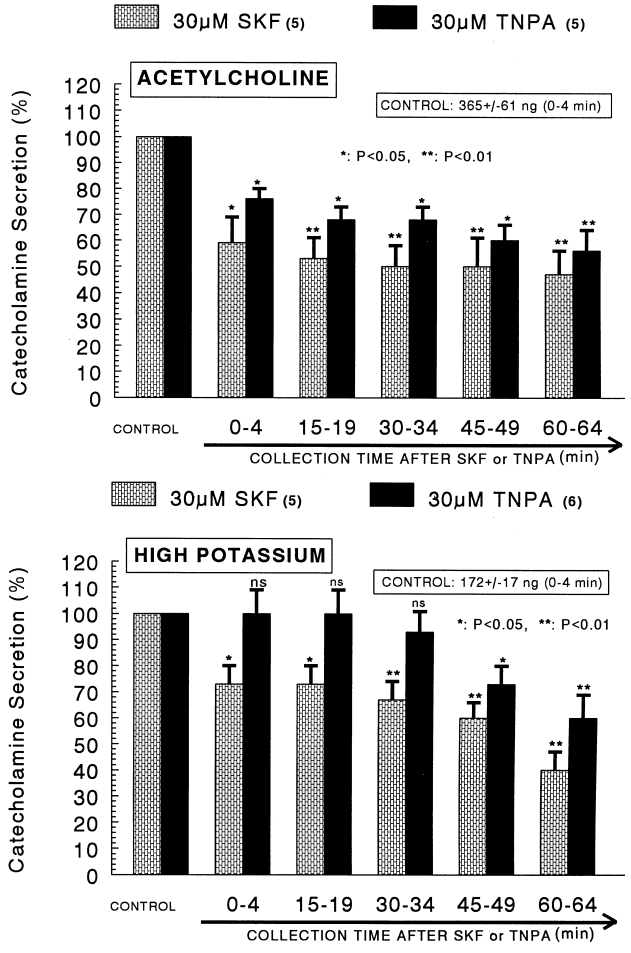

Fig. 1. Time course effect of SKF81297 (SKF) and R-(-)TNPA(TNPA) on the secretory responses of catecholamines (CA) from the isolated perfused rat adrenal glands evoked by acetylcholine (ACh, Upper) and by high K^+ (Lower). CA secretion by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 30 μM of SKF or TNPA for 60 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). Abscissa: collection time of perfusate (min). Statistical difference was obtained by comparing the corresponding control (CONT) with each concentration-pretreated group of SKF81297. Pefusates induced by ACh and high K^+ were collected for 4 minutes, respectively. *$p<0.05$, **$p<0.01$. ns: Statistically not significant.

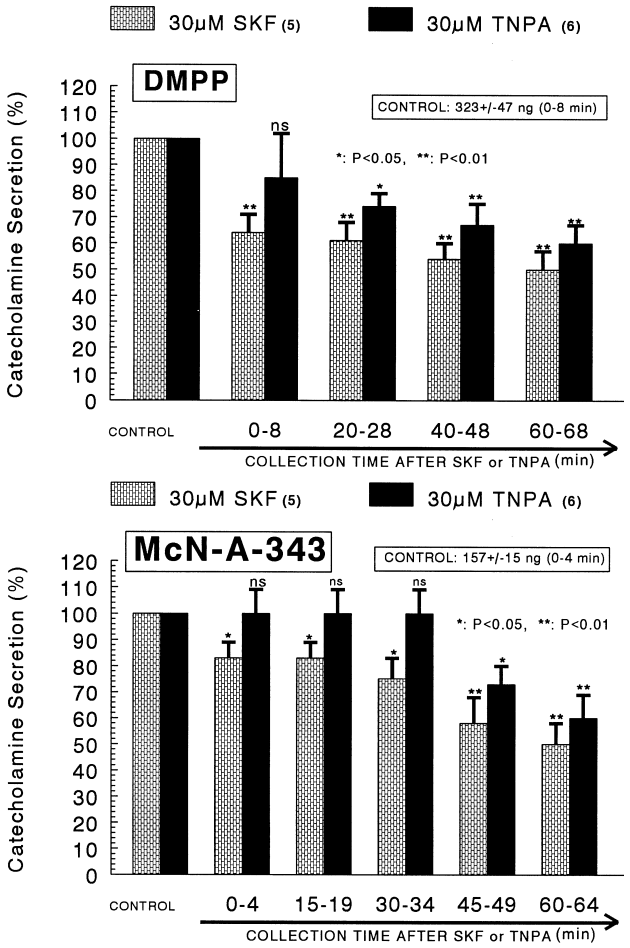

Fig. 2. Time course effect of SKF81297 (SKF) and R-(-)TNPA(TNPA) on the CA secretory responses from the isolated perfused rat adrenal glands evoked by DMPP (Upper) and McN-A-343 (Lower). The CA secretory responses by the perfusion of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced after preloading with 30 μM of SKF or TNPA for 60 min, respectively. Pefusates induced by DMPP and McN-A-343 were collected for 8 and 4 minutes, respectively. Other legends are the same as in Fig. 1. *$p<0.05$, **$p<0.01$. ns: Statistically not significant.

(100%), although they were not affected for 0～34 min period (Fig. 2-Lower).

Effects of SKF81297 and R-(-)-TNPA on the CA secretion evoked by Bay-K-8644 and cyclopiazonic acid 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. 3 illustrates the inhibitory effects of SKF81297 and R-(-)-TNPA on the CA secretory responses evoked by Bay-K-8644 abd cyclopiazonic acid. 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 (3×10^{-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. 3 (Upper). Also, R-(-)-TNPA (3×10^{-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. 3-Upper).

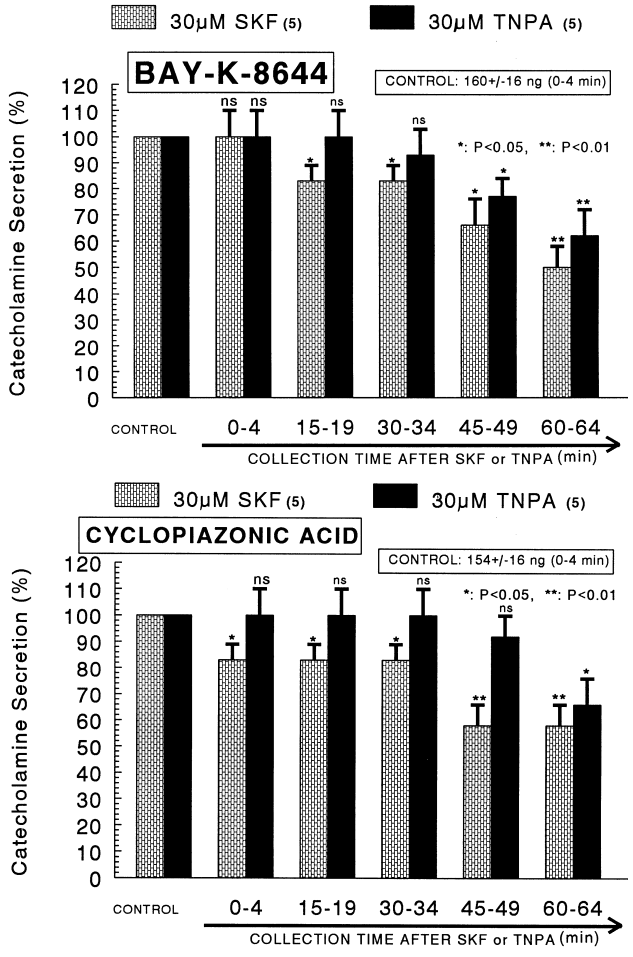

Fig. 3. Time course effect of SKF81297 (SKF) and R-(-)TNPA(TNPA) on the CA release from the rat adrenal glands evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower). Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with 30 μM of SKF or TNPA for 60 min, respectively. Other legends are the same as in Fig. 1. *$p<0.05$, **$p<0.01$. ns: Statistically not significant.

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. 3 (Lower), 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 nhibited 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. 3-Lower).

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. 1~3, 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, SKF81297, and the dopamine D_1 receptor antagonist, 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 the 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 (5.32×10^{-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 (3×10^{-6} M) and S(-)-raclopride (3×10^{-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

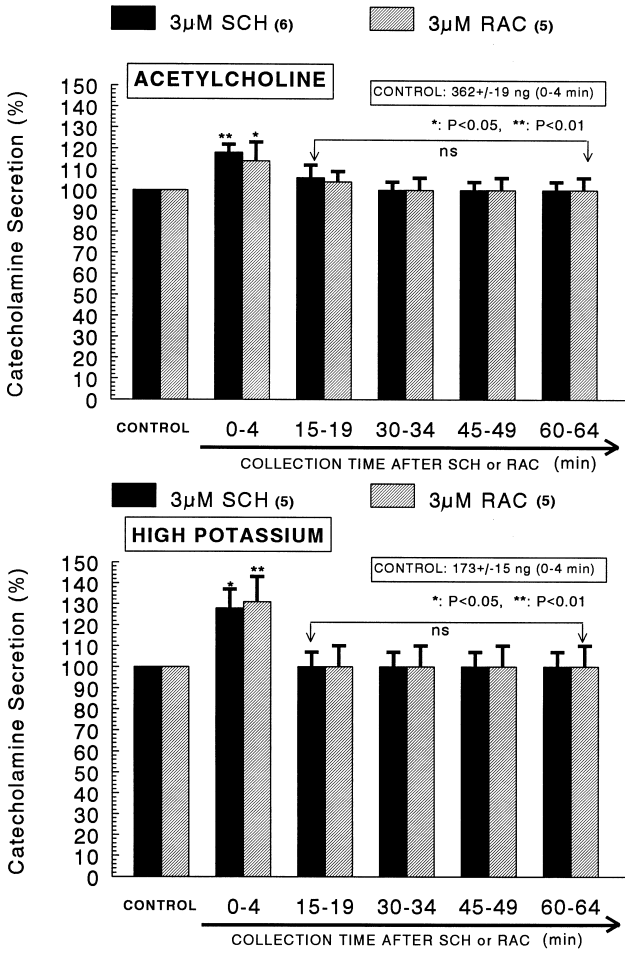

Fig. 4. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the CA release evoked from the isolated perfused rat adrenal glands by acetylcholine (Upper) and high K^+ (Lower). CA secretion by a single injection of Ach (5.32×10^{-3} M) or high K^+ (5.6×10^{-2} M) was induced "BEFORE (CONTROL)" and " AFTER" preloading simultaneously with 3 μM SCH or 3 μM RAC for 60 min, respectively. Other legends are the same as in Fig. 1. *$p<0.05$, **$p<0.01$. ns: Statistically not significant.

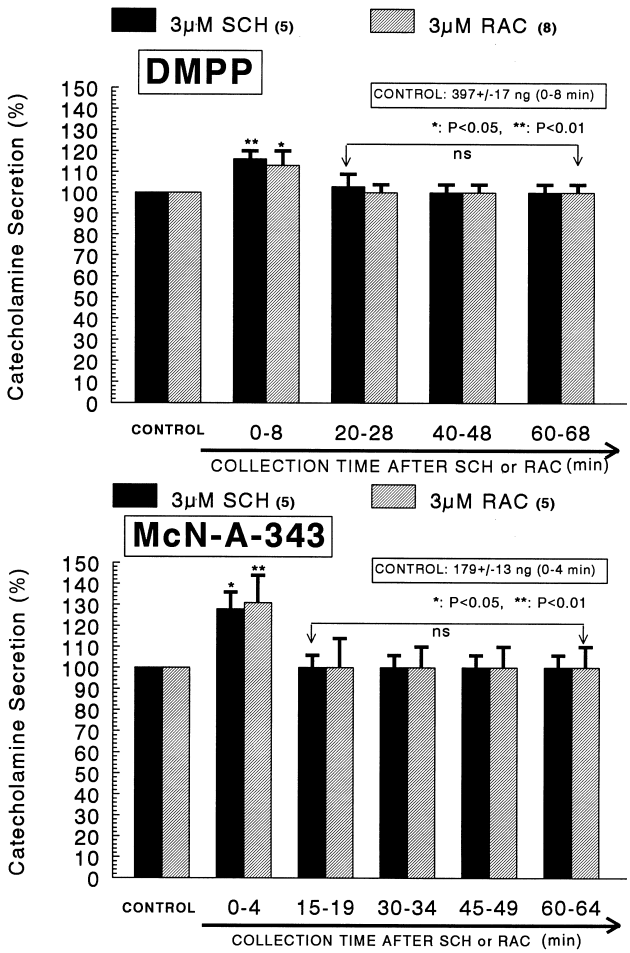

Fig. 5. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the CA release from the isolated perfused rat adrenal glands evoked by DMPP (Upper) and McN-A-343 (Lower). The CA secretory responses by the perfusion of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min and 4 min at 20 and 15 min intervals were induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 3 μM SCH or 3 μM RAC for 60 min, respectively. Other legends are the same as in Fig. 1. *$p<0.05$, **$p<0.01$. ns: Statistically not significant.

release (Fig. 4-Upper). High potassium (56 mM KCl), a direct membrane-depolarizing agent, stimulates the CA secretion (173±15 ng, 0~4 min). In the present work, high K^+ (5.6×10^{-2} M)-evoked CA release in the presence of R(+)-SCH23390 (3×10^{-6} M) and S(-)-raclopride (3×10^{-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. 4 (Lower). DMPP (10^{-4} M), a selective nicotinic receptor, evoked a sharp increase in CA secretion (397±17 ng, 0~8 min). As shown in Fig. 5 (Upper), DMPP (10^{-4} M)-stimulated CA secretion following the loading with R(+)-SCH23390 (3×10^{-6} M) and S(-)-raclopride (3×10^{-6} M) was potentiated by 116% (n=5) and 113% (n=8) of the corresponding control secretion, respectively, which was also the peak release o nly at first 0~8 min without any alteration at 20~68 min period. As illustrated in Fig. 5 (Lower), 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 (3×10^{-6} M) and S(-)-raclopride (3×10^{-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 (3×10^{-6} M) and S(-)-raclopride (3×10^{-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. 6 (Upper).

As depicted in Fig. 6 (Lower), in the presence of R(+)-SCH23390 (3×10^{-6} M) and S(-)-raclopride (3×10^{-6} M), cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also potentiated to 125% (n=5) and 128% (n=5) of the corres-

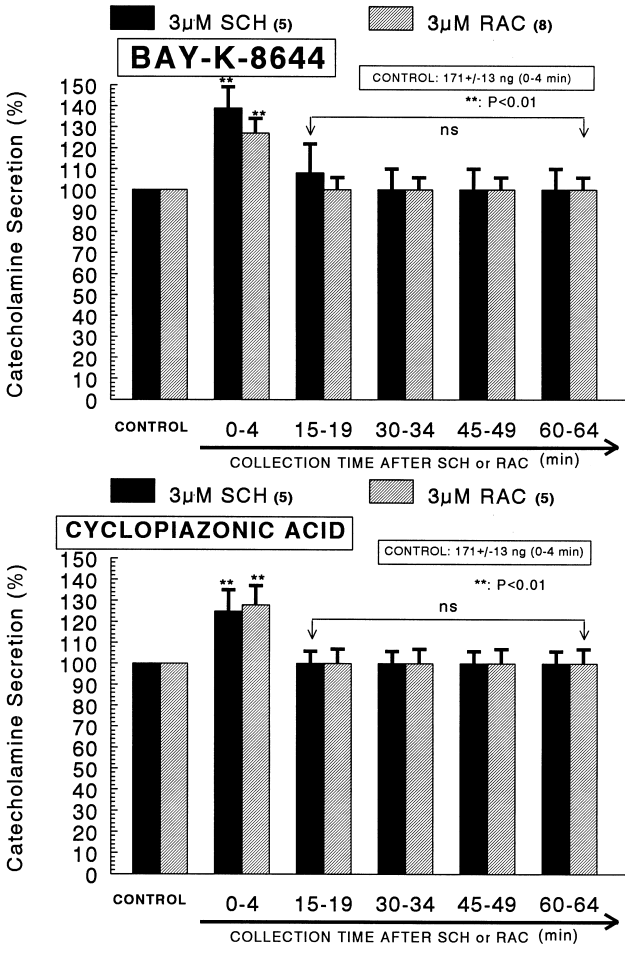

Fig. 6. Time course effect of R(+)-SCH23390 (SCH) and S(-)-raclopride (RAC) on the CA release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals "BEFORE (CONTROL)" and " AFTER" preloading simultaneously with 3 μM SCH or 3 μM RAC for 60 min, respectively. Other legends are the same as in Fig. 1. **p$<$0.01. ns: Statistically not significant.

ponding 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.

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.

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 (Artalejo et al., 1990) showed specific binding of the rhodamine conjugate of the D_1 antagonist SCH-23390 to almost all of the cells in cultured chromaffin cell. 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 adenylyI cyclase is so weak that it is undetectable in the assay (Dahmer and Senogles, 1996). There are some reports suggesting that there are R(+)-SCH23390 binding sites that are not linked to adenylyI 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 D_1-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 feedback 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 D_1-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. 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. In terms of these findings, it is plausible that dopaminergic D_1 receptors exist on the rat adrenomedullary chromaffin cells.

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 immunohistochemical 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 the present work, the pretreatment of S-(-) raclopride, a selective dopaminergic D_2 antagonist, enhanced the CA secretory responses 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. 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, 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 of 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 also 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 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 D_1- and D_2-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 D_1 dopamine receptor (Artalejo et al., 1991), the conditions that showed facilitation were repeated in the presence of SCH-23390, a D_1 antagonist. While this agent blocked facilitated release in a dose-dependent manner, facilitated release was unaffected by the D_2 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 D_1 receptor sites (Dahmer and Senogles, 2000). Because of this, this facilitation seems to be probably due to this receptor as a "D_1-like receptor." Second, the assay used involves the competition between released epinephrine and the antagonist. Further evidence that a D_1-like receptor is involved in facilitation was obtained with the D_1 agonist, SKF-38393 (Villanueva and Wightman, 2007). 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).

In conclusion, 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.

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