Korean J Physiol Pharmacol
Vol 10: 273-282, October, 2006

R-(-)-TNPA, a Dopaminergic D_2 Receptor Agonist, Inhibits Catecholamine Release from the Rat Adrenal Medulla

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The aim of the present study was to investigate the effects of R-(-)-2,10,11-trihydroxy-N-propylnoraporphine [R-(-)-TNPA], a selective agonist of dopaminergic D_2 receptor and S(-)-raclopride, a selective antagonist of dopaminergic D_2 receptor, on the secretion of catecholamines (CA) evoked by cholinergic stimulation and membrane-depolarization in the isolated perfused model of the rat adrenal gland, and also to establish its mechanism of action. R-(-)-TNPA (10~100 μM) perfused into an adrenal vein for 60 min produced dose- and time-dependent inhibition in CA secretory responses evoked by ACh (5.32 mM), high K^+ (56 mM), DMPP (100 μM) and McN-A-343 (100 μM). R-(-)-TNPA itself did also fail to affect basal CA output. Also, in adrenal glands loaded with R-(-)-TNPA (30 μM), the CA secretory responses evoked by Bay-K-8644 (10 μM), an activator of L-type Ca^{2+} channels and cyclopiazonic acid (10 μM), an inhibitor of cytoplasmic Ca^{2+}-ATPase were also inhibited. However, S(-)-raclopride (1~10 μM), given into an adrenal vein for 60 min, enhanced the CA secretory responses evoked by ACh, high K^+, DMPP and McN-A-343 only for the first period (4 min), although it alone has weak effect on CA secretion. Moreover, S(-)-raclopride (3.0 μM) in to an adrenal vein for 60 min also augmented the CA release evoked by BAY-K-8644 and cyclopiazonic acid only for the first period (4 min). However, after simultaneous perfusion of R-(-)-TNPA (30 μM) and S(-)-raclopride (3.0 μM), the inhibitory responses of R-(-)-TNPA (30 μM) on the CA secretion evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644, and cyclopiazonic acid were significantly reduced. Taken together, these experimental results suggest that R-(-)-TNPA greatly inhibits the CA secretion from the perfused rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization, but S(-)-raclopride rather enhances the CA release by them. It seems that this inhibitory of R-(-)-TNPA may be mediated by stimulation of inhibitory dopaminergic D_2 receptors located on the rat adrenomedullary chromaffin cells, while the facilitatory effect of S(-)-raclopride is due to the blockade of dopaminergic D_2 receptors, which are relevant to extra- and intracellular calcium mobilization. Therefore, it is thought that dopaminergic D_2 receptors may be involved in regulation of CA release in the rat adrenal medulla.

Key Words: R-(-)-TNPA, S(-)-raclopride, Catecholamine secretion, Adrenal meduula, Dopaminergic D_2 receptors

INTRODUCTION

The presence of dopamine receptors in adrenomedullary chromaffin cells has been widely reported. Previous studies suggest that, in adrenal medulla and in sympathetic neurons, dopamine (DA) receptors belonging to the D-2 family inhibit CA secretion; in particular, in different species, mature chromaffin cells express dopaminergic receptors of the D-2 subfamily (Gonzales et al, 1986; Mannelli et al, 1990; Amenta & Ricci, 1995; Dahmer and Senogles, 1996a) which negatively modulates the CA secretory process triggered by stimulation of the nicotinic cholinergic receptor (Artalejo et al, 1985; Quik et al, 1987; Bigornia et al, 1888; 1990; Lim et al, 1994). Furthermore, Damase-Michel and his colleagues (1999) have also reported that dopaminergic inhibition of CA secretion from adrenal medulla of the dog is mediated by D_2-like but D_1-like dopaminergic receptors. At the time, these data were interpreted as evidence that D_2 dopamine receptors on the cells inhibited CA release. In contrast to the inhibitory results of D_2 dopamine receptor agonists, the several studies have shown that quinpirole and other dopamine D_2-like receptor agonists acutely elevate adrenal dopamine levels (Kujacic et al, 1990; 1991). Kujacic and Carlsson (1995) have also found that peripherally located dopamine D_2-like receptors are capable of acutely stimulating not only the synthesis of CA, but also the release of epinephrine from adrenals in the conscious

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ABBREVIATIONS: R-(-)-TNPA, R-(-)-2, 10, 11-trihydroxy-N-propyl-noraporphine hydrobromide; CA, catecholamine; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; DAT, dopamine transporter; VDCCs, voltage-dependent calcium channels; VMAT, vesicular monoamine transporter; DHP, dihydropyridine.

rat. Both quinpirole and dopamine significantly enhanced the rate of DOPA accumulation in the rat adrenals, indicating stimulation of adrenal tyrosine hydroxylase (Kujacic & Carlsson, 1993; 1995). The effect of dopamine was blocked by domperidone, a dopamine D_2 receptor antagonist that penetrates poorly into the central nervous system. In addition, Huettl and his colleagues (1991) have demonstrated that functional dopamine D_2 receptors of the classical type are not existed on isolated bovine chromaffin cells. 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).

On the other hand, Dahmer and Senogles (1996b) 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, D_1-selective agonists are found to inhibit secretagogue-stimulated Na^+ uptake in a cyclic AMP-independent manner (Dahmer & Senogles, 1996b).

As described above, it is clear that there are many controversial reports on the modulatory effect of dopaminergic receptors in CA release from the adrenal medulla. Therefore, the first aim of the present study is to investigate whether dopaminergic D_2 receptors exist on the rat adrenomedullary chromaffin cells. The second aim is to examine whether the activation of dopaminergic D_2 receptors can modify the release of CA from the perfused model of the adrenal gland, and to establish its mechanism of action. The present study was carried out to investigate the effect of R-(−)-TNPA, a selective agonist of dopaminergic D_2 receptors, on CA secretion evoked by cholinergic stimulation amd membrane depolarization from the isolated perfused model of the rat adrenal gland, in comparison with the responses to S(−)-Racropride, a selective antagonist of dopaminergic D_2 receptors.

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.) 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 pre-injection 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 R-(−)-TNPA or S(−)-raclopride on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing R-(−)-TNPA or S(−)-raclopride 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 R-(−)-TNPA or S(−)-raclopride, 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 & 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: 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-di hydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)-pyridine-5-carboxylate (BAY-K8644) (Sigma Chemical Co., U.S.A.), and cyclopiazonic acd, [3-(m-cholro-phenyl-carbamoyl-oxy]-2-butynyl-trimethyl 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 (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

RESULTS

Effect of R-(−)-TNPA on CA secretion evoked by ACh high K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 21±2 ng for 2 min (n=6). Since dopaminergic inhibition of CA secretion from adrenal medulla of the dog is found to be mediated by D_2-like but D_1-like dopaminergic receptors (Damase-Michel et al, 1999), it was attempted initially to examine the effects of R-(−)-TNPA itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, R-(−)-TNPA (10^{-5}~10^{-4} M) itself did not produce any effect on basal CA output from perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of R-(−)-TNPA on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. R-(−)-TNPA was present for 60 minutes after the establishment of the control release.

When ACh (5.32×10^{-2} M) in a volume of 0.05 ml was injected into the perfusion stream, the amount of CA secreted was 342±34 ng for 4 min. However, the pretreatment with R-(−)-TNPA in the range of 10^{-5}~10^{-4} M for 20 min concentration- and time-dependently inhibited ACh-stimulated CA secretion. As shown in Fig. 1 (upper), in the presence of R-(−)-TNPA, ACh-stimulated CA release was inhibited by 50% of the corresponding control release. Also, it has been found that depolarizing agent like KCl stimulates markedly CA secretion (181±17 ng for 0~4 min).

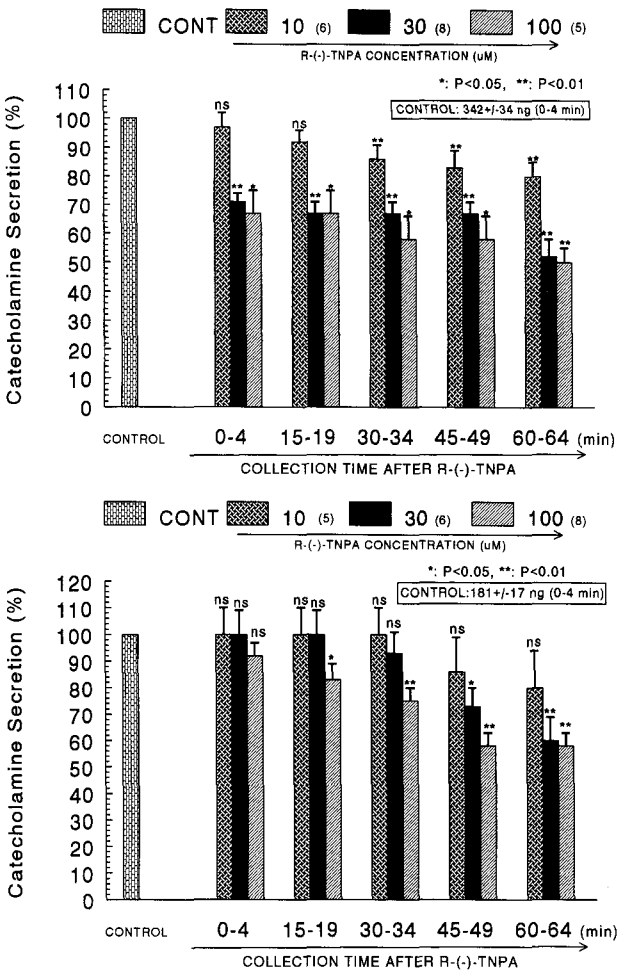

Fig. 1. Dose-dependent effects of R-(−)-TNPA on the secretory responses of catecholamines (CA) evoked by acetylcholine (ACh, **Upper**) and by high K^+ (**Lower**) from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 10, 30, 100 μM of R-(−)-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 R-(−)-TNPA. Pefusates induced by ACh and high K^+ were collected for 4 minutes, respectively. *$p<0.05$, **$p<0.01$. ns: statistically not significant.

Excess K^+ (5.6×10^{-2} M)-stimulated CA secretion after the pretreatment with 10^{-5} M R-(−)-TNPA was not affected for the first 30 min as compared with its corresponding control secretion (100%) (Fig. 1-lower). However, following the pretreatment with higher concentrations of R-(−)-TNPA (3×0^{-5} M and 10^{-4} M), excess K^+ (5.6×10^{-2} M)-stimulated CA secretion was significantly inhibited to 57% of the control after 45 min period, although it was not initially affected by R-(−)-TNPA.

DMPP (10^{-4} M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion (327±40 ng for 0~8

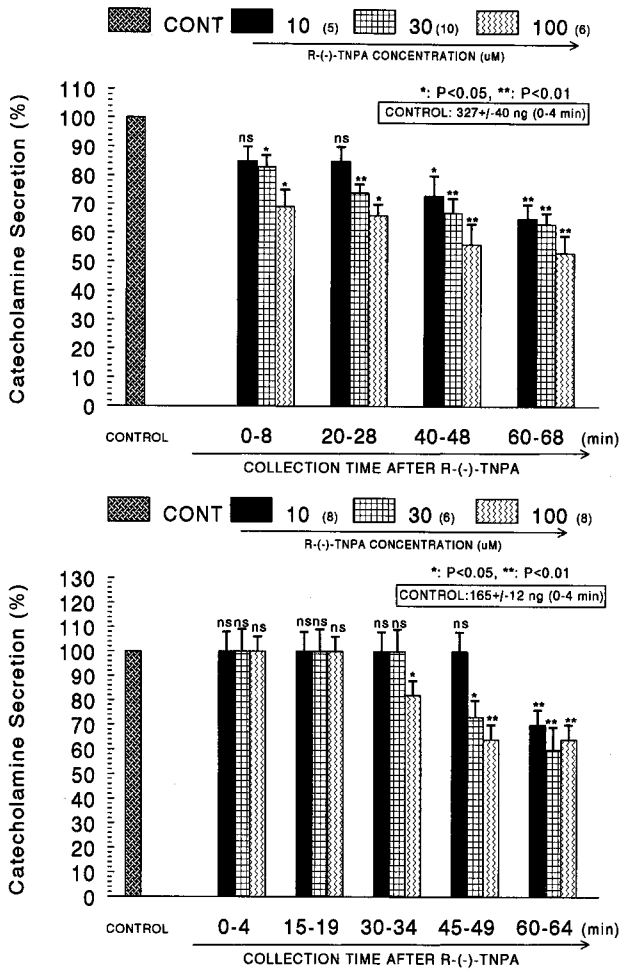

Fig. 2. Dose-dependent effects of R-(−)-TNPA on the secretory responses of catecholamines (CA) evoked by DMPP (**Upper**) and McN-A-343 (**Lower**) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced after preloading with 10, 30, 100 μM of R-(−)-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.

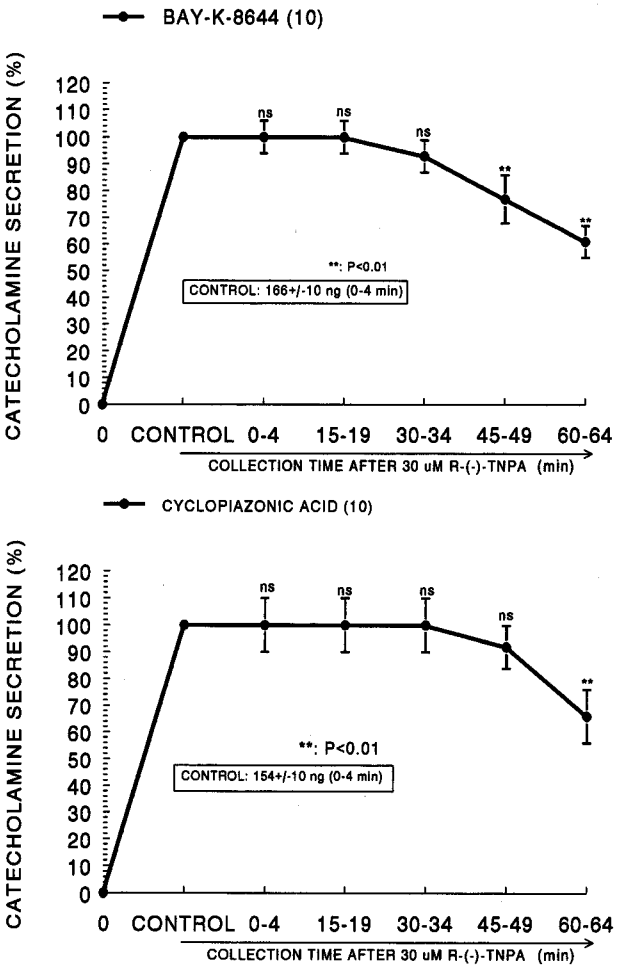

Fig. 3. Effects of R-(−)-TNPA on CA release evoked by Bay-K-8644 (**Upper**) and cyclopiazonic acid (**Lower**) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of R-(−)-TNPA (30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

min). However, as shown in Fig. 2 (upper), DMPP-stimulated CA secretion after pretreatment with R-(−)-TNPA was greatly reduced to 53% of the control release (100%). McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-agonist (Hammer & Giachetti, 1982), perfused into an adrenal gland for 4 min caused an increased CA secretion (165±12 ng for 0~4 min). However, McN-A-343-stimulated CA secretion in the presence of R-(−)-TNPA was markedly depressed to 60% of the corresponding control secretion (100%) as depicted in Fig. 2 (lower).

Effect of R-(−)-TNPA on CA secretion evoked b Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

Since Bay-K-8644 is known to be a calcium channel activator, which enhances basal Ca^{2+} uptake (Garcia et al, 1984) and CA release (Lim et al, 1992), it was of interest to determine the effects of R-(−)-TNPA on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10^{-5} M)-stimulated CA secretion in the presence of R-(−)-TNPA was greatly blocked to 62% of the control except for the early 30 min as compared to the corresponding control release (166±10 ng for 0~4 min) from 5 rat adrenal glands as shown in Fig. 3 (upper).

Cyclopiazonic acid, a mycotoxin from *Aspergillus* and *Penicillium*, has been described as a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Goeger & Riley, 1989; Seidler et al, 1989). The inhibitory action of R-(−)-TNPA on cyclopiazonic acid-evoked CA secretory response was observed as shown in Fig. 3 (lower). However, in the presence of R-(−)-TNPA in 5 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also inhibited to 66% of the control response (154±10 ng for 0~4 min).

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

As shown in Fig. 1 and 2, R-(−)-TNPA significantly inhibited the CA secretory responses evoked by cholinergic stimulation and membrane depolarization form the perfused rat adrenal glands. Therefore, in order to investigate the effect of dopaminergic D_2 receptor antagonist on CA release, it was likely of interest to examine effect of S(−)-raclopride, a selective D_2 antagonist, on CA secretion evoked by by cholinergic stimulation and membrane depolarization from the isolated perfused rat adrenal glands. In order to test the effect of S(−)-raclopride on cholinergic receptor-stimulated CA secretion as well as membrane depolarization-mediated secretion, three concentrations (10^{-5}~10^{-4} M) of S(−)-raclopride were loaded into the adrenal medulla.

In 6 rat adrenal glands, this S(−)-raclopride-evoked CA secretory responses were 22~39 ng (0~60 min), which seemed to be a very weak secretagogue. Therefore, in the subsequent experiments, the dose-dependent effects of S(−)-raclopride on the CA seretory responses evoked by ACh, high K^+, DMPP and McN-A-343 were examined. As illustrated in Fig. 4 and 5, S(−)-raclopride at various concentrations (10^{-6}~10^{-5} μM) produced effective enhancement of CA secretory responses evoked by ACh, high K^+, DMPP and McN-A-343, although it did not at higher concentration (<10 μM).

In the present experiment, ACh (5.32×10^{-3} M)-evoked CA release prior to the perfusion with S(−)-raclopride was

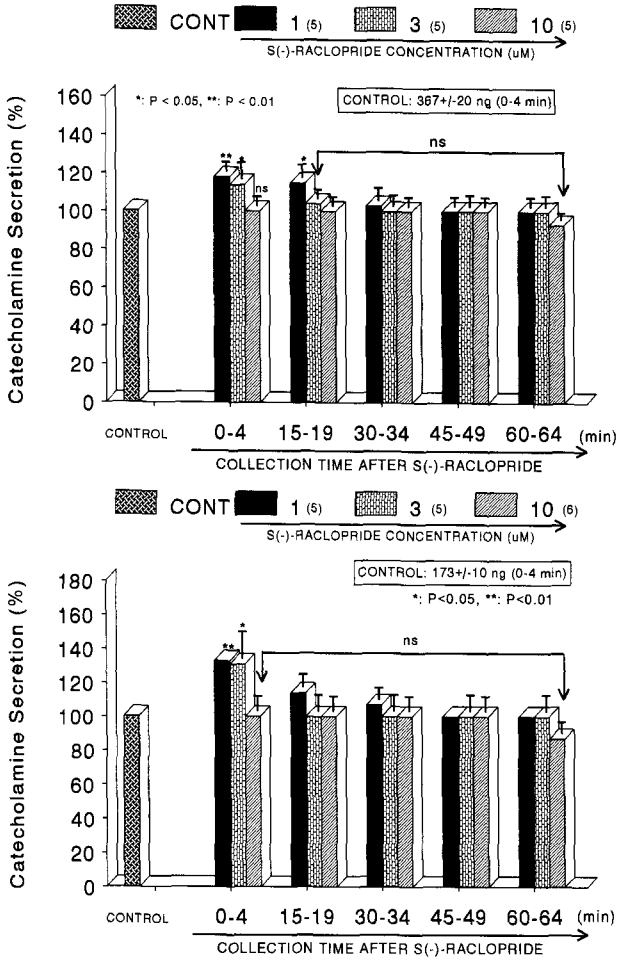

Fig. 4. Effects of S(−)-raclopride on the secretory responses of catecholamines evoked by acetylcholine (**Upper**) and high potassium (**Lower**) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32×10^{-3} M) or high K^+ (5.6×10^{-2} M) was induced "BEFORE (CONTROL)" and "AFTER" preloading with S(−)-raclopride (1~10 μM) for 60 min. Statistical difference was obtained by comparing the corresponding "CONTROL" with each period "AFTER" the intiation of S(−)-raclopride perfusion. Perfusates were collected for 4 minutes at 15 min intervals. Other legends are the same as in Fig. 1. *p<0.05, **p<0.01. ns: statistically not significant.

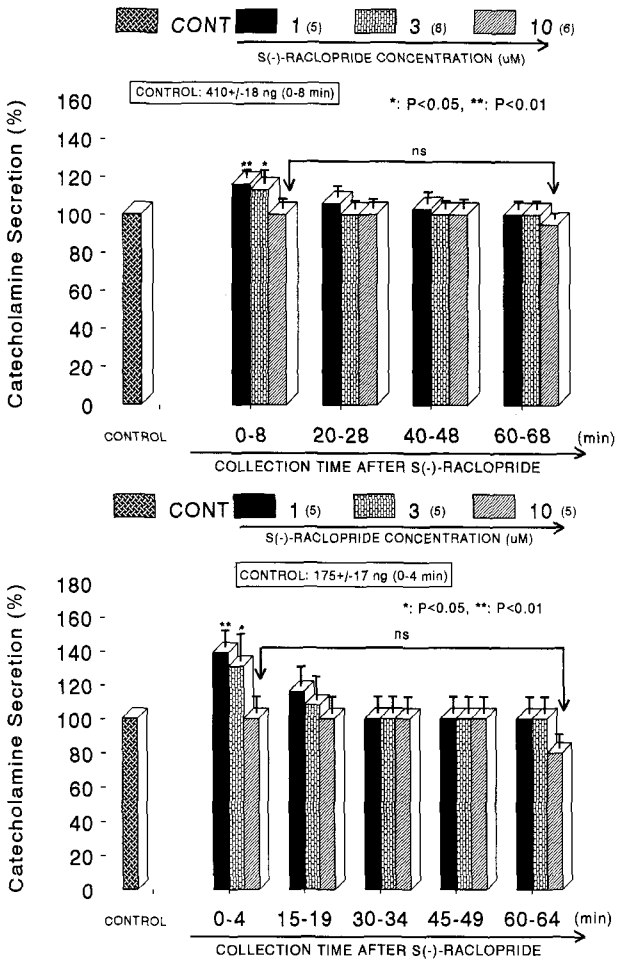

Fig. 5. Effects of S(−)-raclopride on the secretory responses of catecholamines evoked by DMPP (**Upper**) and McN-A-343 (**Lower**) from the isolated perfused rat adrenal glands. CA secretion by perfusion of DMPP (10^{-4} M) or McN-A-343 (10^{-4} M) was induced before (CONTROL) and after perfusion with S(−)-raclopride (1~10 μM) for 60 min. DMPP- and McN-A-343-induced perfusates were collected for 8 and 4 minutes at 20 and 15 min interval, respectively. Other legends are the same as in Fig. 1. *p<0.05, **p<0.01. ns: statistically not significant.

367 ± 20 ng (0~4 min). In the presence of S(−)-raclopride ($10^{-6} \sim 10^{-5}$ μM) for 60 min, it was significantly increased by 118% only at first 0~4 min, but not affected at higher concentration (10^{-5} μM) of S(−)-raclopride. Also, it was never altered at 15~64 min in comparison with the corresponding control release (Fig. 4-upper). High potassium (56 mM KCl), a direct membrane-depolarizing agent, stimulates CA secretion (173 ± 10 ng, 0~4 min). In the present work, high K^+ (5.6×10^{-2} M)-evoked CA release in the presence of S(−)-raclopride (1~10 μM) for 60 min was also enhanced by 133% only at first 0~4 min in comparison to the corresponding control secretion (173 ± 10 ng, 0~4 min) without alteration only at higher concentration (10 μM), as shown in Fig. 4 (lower).

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. As shown in Fig. 5 (upper), DMPP (10^{-4} M)-stimulated CA secretion following the loading with S(−)-raclopride (1~10 μM) was greatly potentiated by 116% compared to the corresponding control secretion (410 ± 18 ng, 0~8 min), which was also the peak release only at first 0~8 min without any alteration only at higher concentration (10 μM). As illustrated in Fig. 5 (lower), McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-receptor agonist (Hammer & Giachetti, 1982), perfused into an adrenal vein for 4 min caused an increased CA secretion to 183 ± 5 ng (0~4 min). However, in the presence of S(−)-raclopride (1~10 μM), McN-A-343-evoked CA secretion was significantly increased by 139% only at first 0~4 min of the corresponding control release (175 ± 17 ng/0~4 min) without any alteration only at higher concentration (10 μM).

Bay-K-8644 (10^{-5} M)-stimulated CA secretion in the presence of S(−)-raclopride was greatly enhanced to 127% only at first 0~4 min of the control of the corresponding

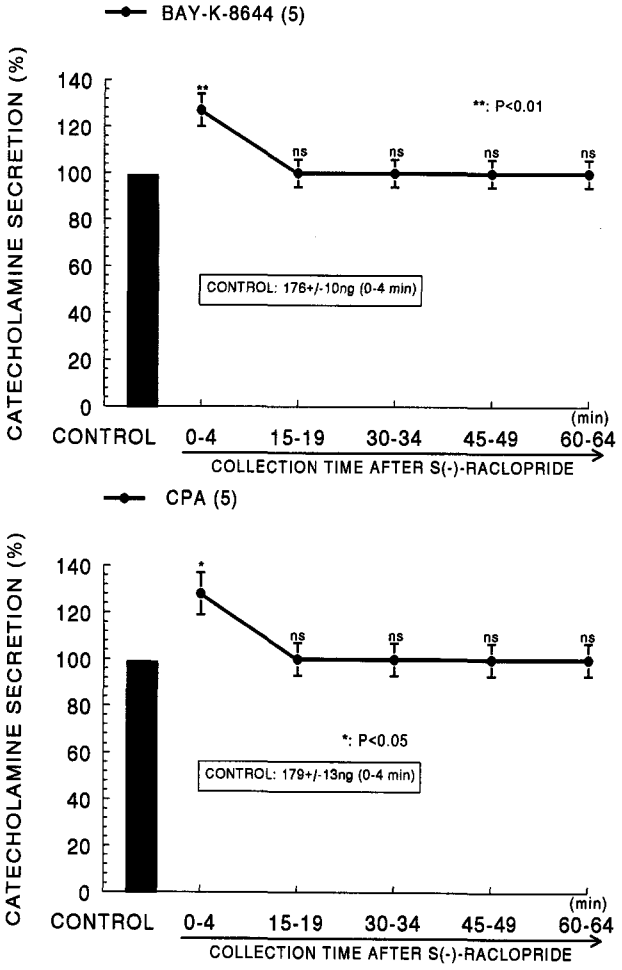

Fig. 6. Effects of S(−)-raclopride on the secretory responses of catecholamines evoked by Bay-K-8644 and cyclopiazonic acid from the isolated perfused rat adrenal glands. CA secretion by perfusion of Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (CPA, 10^{-5} M) for 4 min was induced before (CONTROL) and after perfusion with S(−)-raclopride (3 μM) for 60 min, respectively. Bay-K-8644-induced perfusates were collected for 4 minutes at 15 min interval Other legends are the same as in Fig. 1. *p<0.05, **p<0.01. ns: statistically not significant.

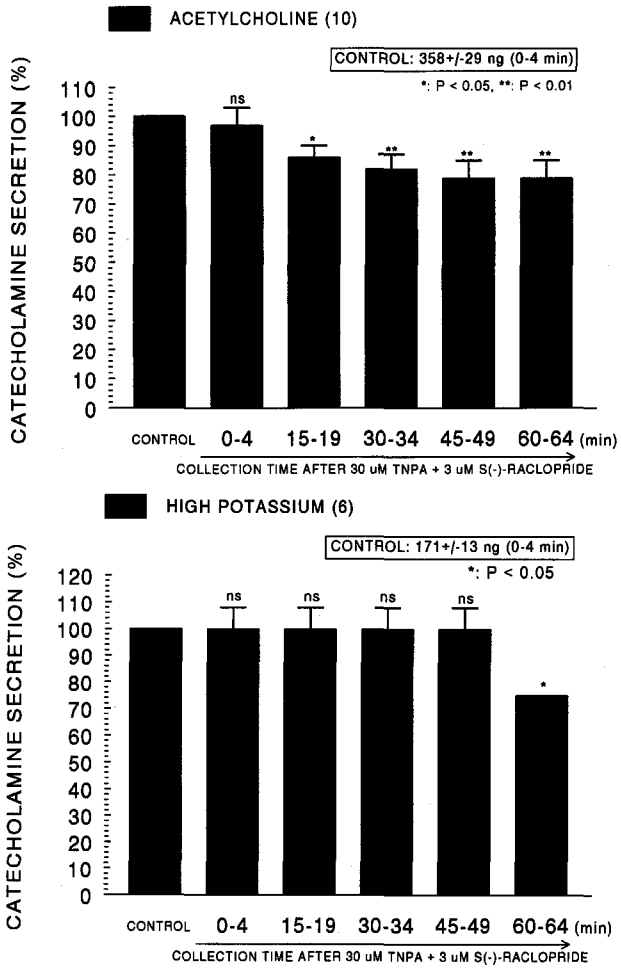

Fig. 7. Effects of R-(−)-TNPA plus S(−)-raclopride on catecholamine release evoked by acetylcholine **(Upper)** and high K^+ **(Lower)** from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32×10^{-3} M) or high K^+ (5.6×10^{-2} M) was induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 30 μM R-(−)-TNPA+3 μM S(−)-raclopride for 60 min, respectively. Other legends are the same as in Fig. 1. *p< 0.05, **p<0.01. ns: statistically not significant.

control release (176±10 ng for 0~4 min) from 5 rat adrenal glands, as shown in Fig. 6 (upper). As depicted in Fig. 6 (lower) in the presence of S(−)-raclopride from 5 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was potentiated to 128% only at first 0~4 min of the control response (179±13 ng for 0~4 min).

Effect of R-(−)-TNPA plus S(−)-raclopride on CA release evoked by ACh, high K^+, DMPP, McN-A-343 BAY-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

It has also been found that, in this study, R-(−)-TNPA inhibits the CA secretory response evoked by cholinergic stimulation in the perfused rat adrenal gland. Therefore, to study the relationship between dopaminergic D_2 receptors and CA release from the rat adrenal glands, the effect of S(−)-raclopride on R-(−)-TNPA-induced inhibitory responses of CA secretion evoked by cholinergic receptor-stimulation as well as membrane depolarization was examined. In the present study.

ACh (5.32 mM)-evoked CA release before perfusion with R-(−)-TNPA plus S(−)-raclopride was 358±29 ng (0~4 min) from 10 rat adrenal glands. In the simultaneous presence of R-(−)-TNPA (30 μM) and S(−)-raclopride (3 μM) for 60 min, it was initially not affected at 0~19 min, but later rather inhibited by 79~86% of the corresponding control release at the period of 15~64 min as illustrated in Fig. 7 (upper). High K^+ (56 mM)-evoked CA release in the presence of R-(−)-TNPA (30 μM) and S(−)-raclopride (3 μM) for 60 min was also not changed for 0~49 min, but later rather inhibited to 75% of the corresponding control release only at the last period of 60~64 min period in comparison to the control secretion (171±13 ng, 0~4 min) from 6 glands (Fig. 7-lower).

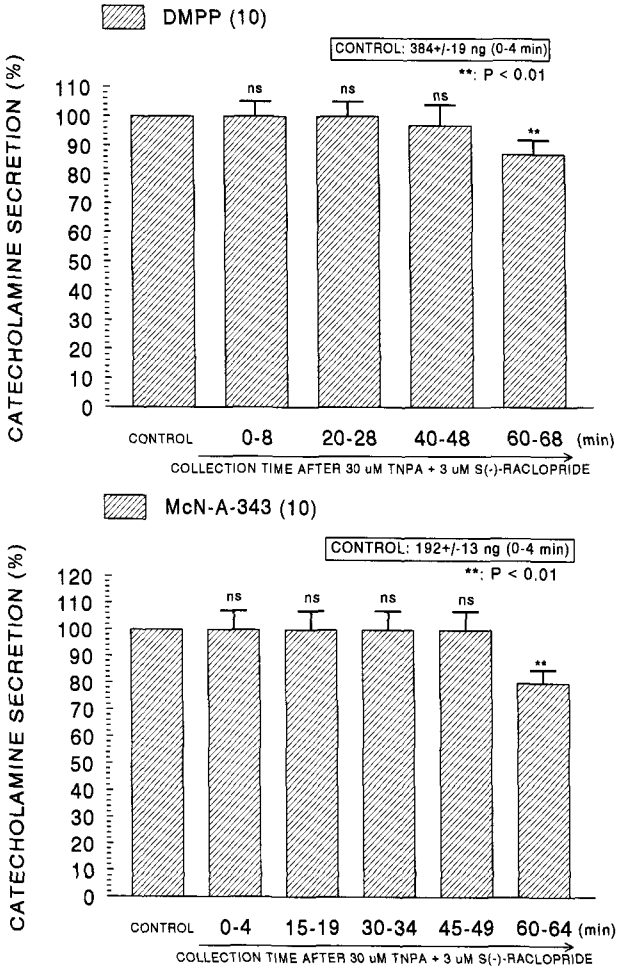

Fig. 8. Effects of R-(−)-TNpA plus S(−)-raclopride on catecholamine release evoked by DMpp (**Upper**) and McN-A-343 (**Lower**) from the isolated perfused rat adrenal glands. The CA secretory responses by the perfusion of DMpp (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min and 4 min at 20 and 15 min intervals were induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 30 μM R-(−)-TNPA+3 μM S(−)-raclopride for 60 min, respectively. Other legends are the same as in Fig. 1. *p<0.05, **p<0.01. ns: statistically not significant.

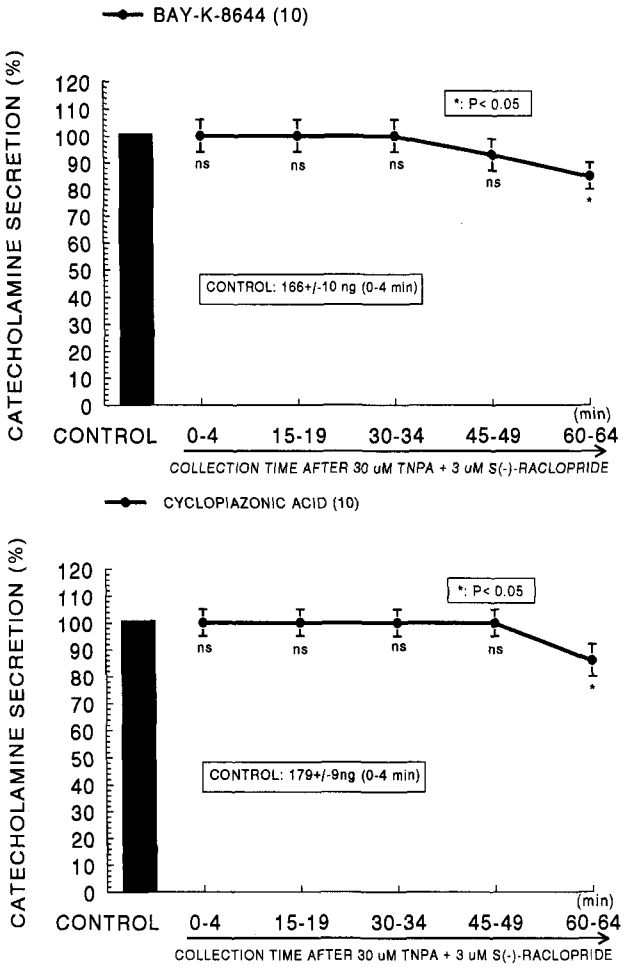

Fig. 9. Effects of R-(−)-TNPA plus S(−)-raclopride on catecholamine release evoked by Bay-K-8644 (**Upper**) and cyclopiazonic acid (**Lower**) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 30 μM R-(−)-TNPA+3 μM S(−)-raclopride for 60 min, respectively. Other legends are the same as in Fig. 1. *p<0.05. ns: statistically not significant.

As shown in Fig. 8 (upper), DMPP-evoked CA release prior to the perfusion with R-(−)-TNPA and S(−)-raclopride was 384±19 ng (0~8 min). The simultaneous perfusion of R-(−)-TNPA and S(−)-raclopride for 60 min no longer inhibited DMPP-evoked CA release for the period of 0~48 min from 10 experiments while later rather depressed to 87% of the control release only at the last period of 60~68 min. Moreover, in the presence of R-(−)-TNPA (30 μM) and S(−)-raclopride (3 μM), the CA secretory response evoked by McN-A-343 (10^{-4} M for 2 min) was also not affected for 0~49 min, but later rather inhibited to 80% of the corresponding control release (192±13 ng, 0~4 min) only at the last period of 60~64 min period from 10 glands, as shown in Fig. 8 (lower).

As shown in Fig. 9, the simultaneous perfusion of R-(−)-TNPA (30 μM) and S(−)-raclopride (3 μM) for 60 min no longer inhibited the CA release evoked by Bay-K-644 and cyclopiazonic acid for the period of 0~49 min from 10 experiments, but later rather depressed to 85% and 86% of the control release only at the last period of 60~64 min in comparison to their corresponding control responses (166± 16 ng/0~4 min and 179±9 ng/0~4 min), respectively.

DISCUSSION

The current experimental results suggest that R-(−)-TNPA inhibits the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization, but S(−)-raclopride rather enhances the CA release by them. It seems that this inhibitory of R-(−)-TNPA may be mediated by stimulation of inhibitory dopaminergic D_2 receptors located on the rat adrenomedullary chromaffin cells, while the facilitatory effect of S(−)-raclopride is due to the blockade of dopaminergic D_2 receptors, which are relevant to extra- and intracellular calcium mobilization. In support of this idea, 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, and that this inhibitory effect was greatly depressed in the presence of S(−)-raclopride, a selective dopaminergic D_2 antagoist strongly suggest that R-(−)-TNPA causes an inhibitory effect on the CA secretion through the the activation of the the inhibitory dopaminergic D_2 receptors located on the rat adrenomedullary chromaffin cells.

It has been shown that the chromaffin cell membrane of the cat adrenal medulla contains a dopaminergic receptor that modulates the CA secretory process triggered by stimulation of the nicotinic cholinoceptor (Artalejo et al, 1985). The fact that dopamine is released in measurable amounts, together with EP and NE from the perfused cat adrenal glands in response to nicotinic stimulation, favors a role for this dopaminergic in modulating CA release from the chromaffin cells. It has also been found that the presence of D_2 dopamine receptors on adrenal chromaffin cells in demonstrated in several studies by radioligand binding methods (Gonzalez et al, 1986; Lyon et al, 1987; Quik 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 a., 1989). 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 reversed the R-(−)-TNPA-induced inhibition of CA secretory process evoked by ACh, high K^+ and DMPP. 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 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 also inhibits these secretagoues-evoked CA release (Choi, 2004). Consistently with the present results, dopaminergic inhibitory effects in other systems were found to

be mediated specifically by the D_2-receptor subtype (De Vliefer et al, 1985; Memo 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 is mediated by D_2-like but not D_1-like dopaminergic receptors (Damase-Michel et al, 1999).

In the present investigation, the finding that R-(−)-TNPA time- and concentration-dependently inhibited 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 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 R-(−)-TNPA produces the inhibitory modulation of adrenal CA secretion at least partly by inhibition of calcium channel currents through stimulation of the inhibitory dopaminergic 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 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 so far 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 dopaminergic D_2-receptor-mediated inhibition of CA secretion.

In the present study, 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 (Goeger & Riley, 1989; Seidler et al, 1989). Therefore, it is thought that the inhibitory effect of 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_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, R-(−)-TNPA produced time- and concentration-dependently inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests new other concept that 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 in 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 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_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). 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 frequencies of the sectioned splanchnic nerve. This result demonstrates 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).

On the other hand, it has 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 cyclic AMP 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. 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 CA release response through a negative feedback loop that uses cyclic AMP as a second messenger. In addition, D_1-like receptors have been reported to inhibit secretion (Schoors et al, 1991); however, such a function for members of the D_1 family of dopamine receptors is controversial.

In conclusion, taken together, these experimental results suggest that R-(−)-TNPA inhibits the CA secretion from the perfused rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization, but S(−)-raclopride rather enhances the CA release by them. It seems that this inhibitory of R-(−)-TNPA may be mediated by stimulation of inhibitory dopaminergic D_2 receptors located on the rat adrenomedullary chromaffin cells, while the facilitatory effect of S(−)-raclopride is due to the blockade of dopaminergic D_2 receptors, which are relevant to extra- and intracellular calcium mobilization. Therefore, it is concluded that the presence of the dopaminergic D_2 receptors may be involved in regulation of CA release in the rat adrenal medulla.

ACKNOWLEDGEMENT

This study was supported partly by a research grant from Chosun University, Gwangju, Korea (2005).

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