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Influence of SKF81297 on Catecholamine Release from the Perfused Rat Adrenal Medulla

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The aim of the present study was to investigate the effects of 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF81297), a selective agonist of dopaminergic D_1 receptor, on the secretion of catecholamines (CA) evoked by cholinergic stimulation and membrane-depolarization in the isolated perfused rat adrenal gland, and also to elucidate the mechanism involved. SKF81297 (10 ~ 100 μM) perfused into an adrenal vein for 60 min produced dose- and time-dependent inhibition of CA secretory responses evoked by ACh (5.32 mM), high K^+ (56 mM), DMPP (100 μM) and McN-A-343 (100 μM). Also, in adrenal glands loaded with SKF81297 (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, in the presence of the dopamine D_1 receptor antagonist, (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (SCH23390, 3 μM), which is a selective antagonist of dopaminergic D_1 receptor, the inhibitory responses of SKF81297 (30 μM) on the CA secretion evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644, and cyclopiazonic acid were significantly reduced. Collectively, these experimental results suggest that SKF81297 inhibits the CA secretion from the rat adrenal medulla evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization. This inhibitory of SKF81297 seems to be mediated by stimulation of dopaminergic D_1 receptors located on the rat adrenomedullary chromaffin cells, which are relevant to extra- and intracellular calcium mobilization. Therefore, it is thought that the presence of the dopaminergic D_1 receptors may be involved in regulation of CA release in the rat adrenal medulla.

Key Words: SKF81297, SCH23390, Dopaminergic D_1 receptors, Adrenal medulla, Catecholamine secretion

INTRODUCTION

Artalejo and his coworkers (1990) have identified D_1 dopaminergic receptors on bovine chromaffin cells by fluorescence microscopy. They have also found that stimulation of the D_1 receptors facilitates Ca^{2+} current in the absence of pre-depolarization or repetitive activity, and that activation by D_1 agonists is mediated by cyclic AMP and protein kinase A. The facilitation of Ca^{2+} channels by dopamine in bovine chromaffin cells may form the basis of a positive feedback loop mechanism for CA secretion. There is also the view that stimulation of peripherally located dopamine D_2-like receptors can enhance the rate of adrenal catecholamine synthesis in rat adrenal gland by stimulating the activity of tyrosine hydroxylase (Kujacic and Carlsson, 1995).

On the other hand, Huettl and his colleagues (1991) demonstrated that functional dopamine D_2 receptors of the classical type do not exist 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). Moreover, it has been suggested that bipotential cells obtained from a newborn rat adrenal medulla express both isoforms of the D-2 receptor, while D-3 receptor and D-4 receptor messenger RNAs (mRNAs) are not present (Sigala et al, 2000). However, in contrast to these findings, Dahmer and Senogles (1996) have observed that the D_1-selective agonist, 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 were found to inhibit secretagogue-stimulated Na^+ uptake in a cyclic AMP-independent manner (Dahmer and Senogles, 1996). It has al-

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ABBREVIATIONS: CA, catecholamine; ACh, acetylcholine; SKF, 6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (SCH23390; DMPP, 1.1-dimethyl-4-phenyl piperazinium iodide; BAY-K-8644, ethyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5- carboxylate; McN-A-343, 4-(N-[3-Chlorophenyl]carbamoyloxy)-2-butynyltrimethyl ammonium chloride.

so been demonstrated that apomorphine dose-dependently inhibits CA secretion induced by cholinergic receptor stimulation and also by membrane depolarization from the isolated perfused rat adrenal gland (Lim et al, 1994).

Thus, it is clear that there are still many controversial reports on the modulating effects of dopaminergic D_1-receptors on the CA release from the adrenal medulla. The purpose of the present study was to investigate whether the activation of dopaminergic D_1 receptors can modify the release of CA from the perfused model of the adrenal gland. To this end, the present study was undertaken to examine the role of dopamine D_1 receptors in the CA release from the isolated perfused rat adrenal glandsm employing 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 (SCH23390), drugs which have widely been 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), and to elucidate the mechanism of its action.

METHODS

Experimental procedure

Sprague-Dawley male rats, weighing 180 to 300 grams, were intraperitoneally anesthetized with thiopental sodium (40 mg/kg). The adrenal gland was isolated by the modification of previous method (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 with a saline-soaked gauge pads, Urine in the 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 the adrenal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/ml) was injected into the 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 the 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 a 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 CAs.

Drug administration

SKF81297 (10~100 μM) and SCH23390 (3 μM) were perfused into an adrenal vein for 60 min. The perfusions of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min 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 the perfusion stream via a three-way stopcock, respectively. In the preliminary experiments, it was found that upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343, and cyclopiazonic acid returned to the pre-injection level in about 4 min, but the responses to DMPP was 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 samples were collected from 4 to 8 min. The amounts secreted in the background sample had 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 on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution, containing SKF81297, for 60 min. Then, the perfusate was collected for a certain period of time (background sample). Then, the medium was changed to the one containing the blocking agent or along with SKF81297, 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 intermediate purification alumina for the reasons described earlier (Wakade, 1981) using a 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 those of the 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 using the 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-tetrahydro-1H-3-benzazepine (SKF81297), (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-benzazepine-7-ol (SCH23390), acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, methyl-1, 4-dihydro-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 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

Effect of SKF81297 on CA secretion from the perfused rat adrenal glands evoked by ACh, high K^+, DMPP and McN-A-343

After the perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 22±3 ng for 2 min (n=6). Since D_1-selective agonists in bovine adrenal chromaffin cells are found to inhibit secretagogue-stimulated Na^+ uptake in a cyclic AMP-independent manner (Dahmer and Senogles, 1996), there was initially an attempt to examine the effects of SKF81297 itself on CA secretion from the perfused model of the rat adrenal glands. However, in the present study, SKF81297 ($10^{-5}\sim10^{-4}$ M) itself did not produce any effect on basal CA output from the perfused rat adrenal glands (data not shown). Therefore, it was decided to investigate the effects of SKF81297 on cholinergic receptor stimulation- as well as membrane depolarization-mediated CA secretion. Secretagogues were given at 15 min-intervals. SKF81297 was present for 60 min after the establishment of the control release.

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 436±74 ng for 4 min. However, the pretreatment with SKF81297 in the range of $10^{-5}\sim10^{-4}$ M for 20 min inhibited ACh-stimulated CA secretion concentration- and time-dependently. As shown in Fig. 1 (Upper), in the presence of SKF81297, CA releasing responses were inhibited by 39% of the corresponding control release. Also, depolarizing agent like KCl markedly stimulated the CA secretion (185 ±20 ng for 0~4 min). High K^+ (5.6×10^{-2} M)-stimulated CA secretion after pretreatment with 10^{-5} M SKF81297 was not affected for the first 30 min as compared with its corresponding control secretion (100%) (Fig. 1, lower panel). However, following the pretreatment with higher concentrations of SKF81297 (3×10^{-5} M and 10^{-4} M), excess K^+ (5.6×10^{-2} M)-stimulated CA secretion was significantly inhibited to 37% of the control after 45 min, although it was not initially affected by SKF81297. DMPP (10^{-4} M), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase of CA secretion (401±53 ng for 0~8 min). However, as shown in Fig. 2 (Upper), DMPP-stimulated CA secretion after pretreatment with SKF81297 was greatly reduced to 42% of the control release (100%). McN-A-343 (10^{-4} M), which is a selective muscarinic M_1-agonist (Hammer and Giachetti, 1982) and was perfused into an adrenal gland for 4 min, caused an increased CA secretion (158±16 ng for 0~4 min). However, McN-A-343-stimulated CA secretion in the presence of SKF81297 was markedly depressed to 42% of the corresponding control secretion (100%), as depicted in Fig. 2 (Lower).

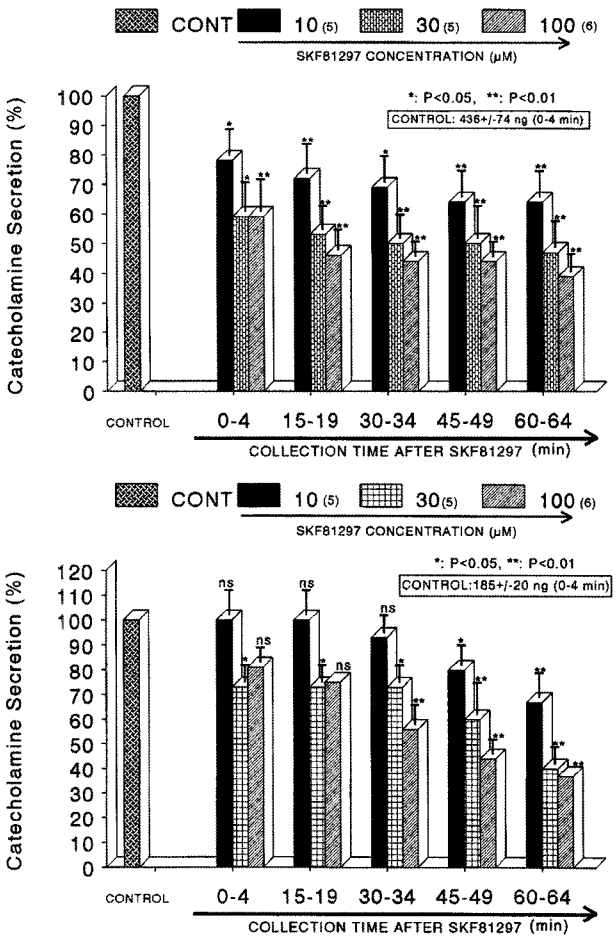

Fig. 1. Dose-dependent effects of SKF81297 on the secretory responses of catecholamines (CA) from the isolated perfused rat adrenal glands evoked by acetylcholine (ACh, Upper) and by high K^+ (Lower). CA secretion by a single injection of ACh (5.32×10^{-3} M) or K^+ (56 mM) in a volume of 0.05 ml was evoked at 15 min intervals after preloading with 10, 30, 100 μM of SKF81297 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 min, respectively. *: $p<0.05$, **: $p<0.01$. ns: Statistically not significant.

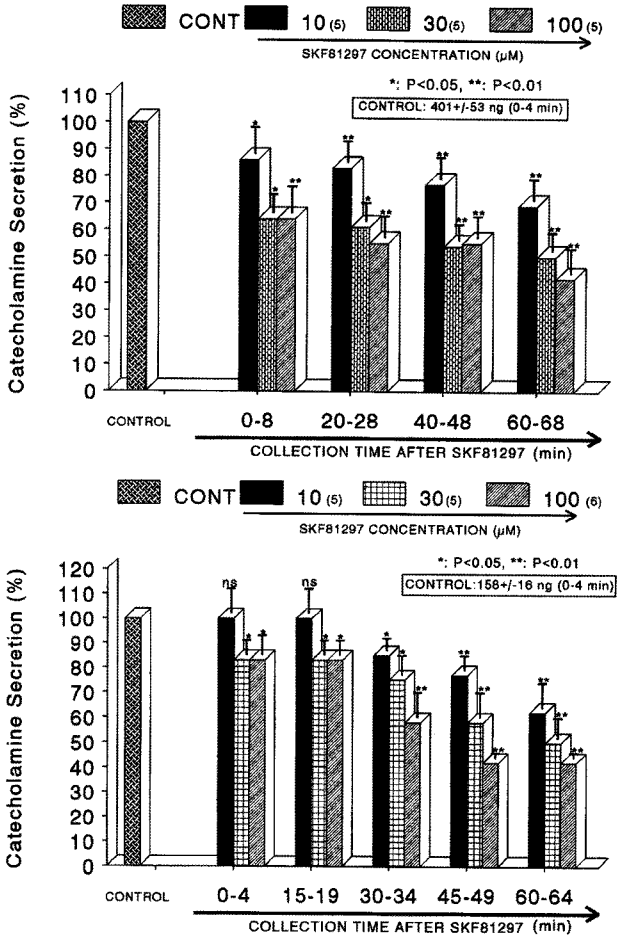

Fig. 2. Dose-dependent effects of SKF81297 on the secretory responses of catecholamines (CA) from the isolated perfused rat adrenal glands evoked by DMPP (Upper) and McN-A-343 (Lower). The CA secretory responses by the perfusion of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min at 20 and 15 min intervals were induced after preloading with 10, 30, 100 μM of SKF81297 for 60 min, respectively. Pefusates induced by DMPP and McN-A-343 were collected for 8 and 4 min, respectively. Other legends are the same as in Fig. 1. *: $p<0.05$, **: $p<0.01$. ns: Statistically not significant.

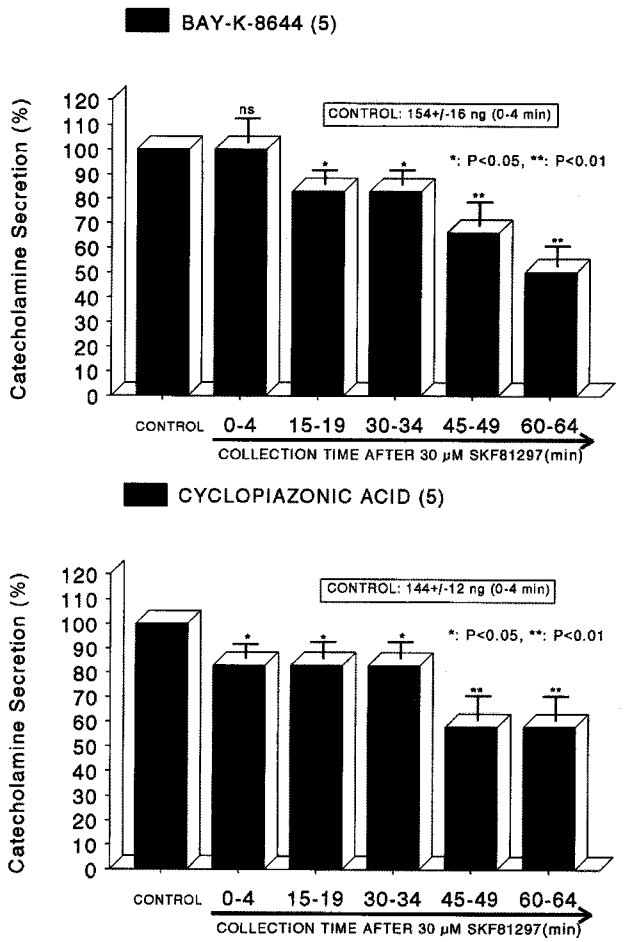

Fig. 3. Effects of SKF81297 on CA release from the rat adrenal glands evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower). Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals after preloading with of SKF81297 (30 μM) for 60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

Effect of SKF81297 on CA secretion from the perfused rat adrenal glands evoked by Bay-K-8644 and cyclopiazonic acid

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 SKF81297 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 SKF81297 was greatly blocked to 50% of the control except for the first 30 min as compared to the corresponding control release (154±16 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 and Riley, 1989; Seidler et al, 1989). As shown in Fig. 3 (Lower), the inhibitory action of SKF81297 on cyclopiazonic acid-evoked CA secretory response was observed. However, in the presence of SKF81297 in 5 rat adrenal glands, cyclopiazonic acid (10^{-5} M)-evoked CA secretion was also inhibited to 58% of the control response (154±16 ng for 0~4 min).

Effect of SKF81297 plus SCH23390 on CA release from the perfused rat adrenal glands evoked by ACh, high K^+, DMPP, McN-A-343, BAY-K-8644 and cyclopiazonic acid

It has also been found in this study that SKF81297 inhibits the CA secretory response in the perfused rat adrenal gland evoked by cholinergic stimulation. Therefore, to study the relationship between dopaminergic D_1 receptors and CA release from the rat adrenal glands in the present study, the effect of SCH23390 on SKF81297-induced inhibitory responses of CA secretion, evoked by cholinergic receptor-stimulation as well as membrane depolarization, was

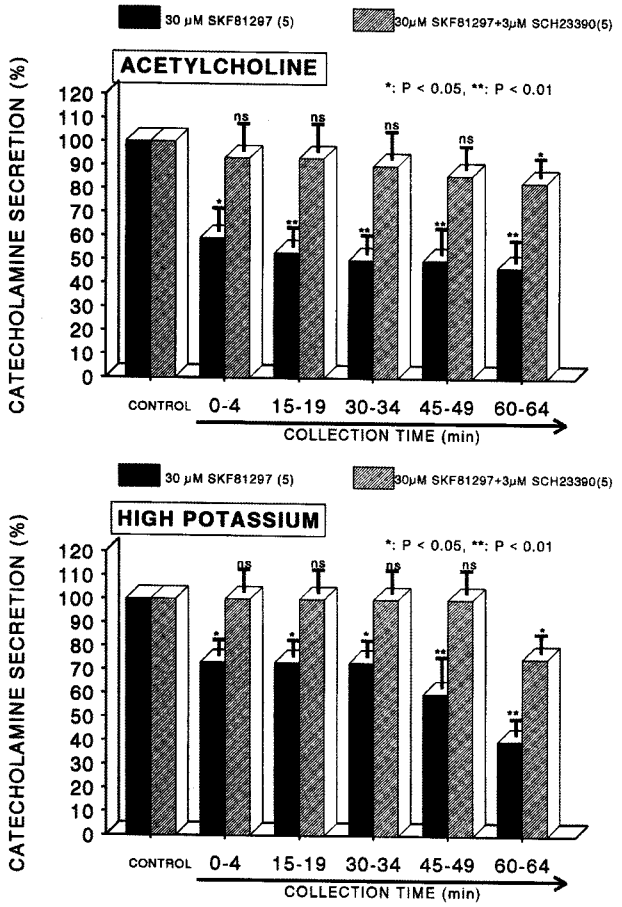

Fig. 4. Effects of SKF81297 plus SCH23390 on catecholamine release evoked from the isolated perfused rat adrenal glands by acetylcholine (Upper) and high K^+ (Lower). CA secretion by a single injection of Ach (5.32×10^{-3} M) or high K^+ (5.6×10^{-2} M) was induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 30 μM SKF81297 + 3 μM SCH23390 for 60 min, respectively. Other legends are the same as in Fig. 1. *: $p<0.05$, **: $p<0.01$. ns: Statistically not significant.

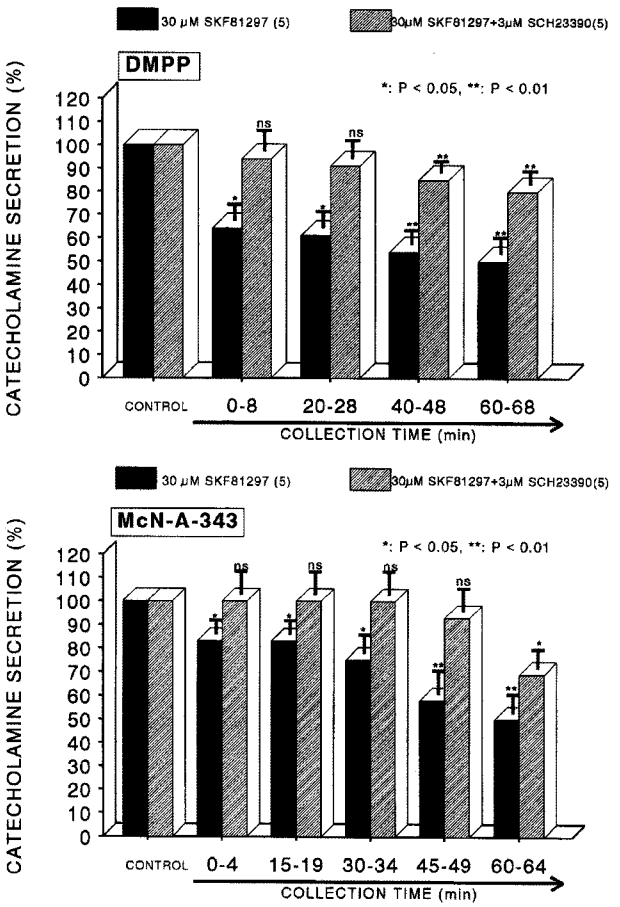

Fig. 5. Effects of SKF81297 plus SCH23390 on catecholamine release from the isolated perfused rat adrenal glands evoked by DMPP (Upper) and McN-A-343 (Lower). The CA secretory responses by the perfusion of DMPP (10^{-4} M) and McN-A-343 (10^{-4} M) for 2 min and 4 min at 20 and 15 min intervals were induced "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 30 μM SKF81297+3 μM SCH23390 for 60 min, respectively. Other legends are the same as in Fig. 1. *: $p<0.05$, **: $p<0.01$. ns: Statistically not significant.

examined. As illustrated in Fig. 4 (Upper, ACh (5.32 mM)-evoked CA release before perfusion with SKF81297 plus SCH23390 was 538±66 ng (0~4 min) from 5 rat adrenal glands. In the simultaneous presence of SKF81297 (30 μM) and SCH23390 (3 μM) for 60 min, it was initially not affected at 0~19 min, howevwr, was inhibited more by 83~86% of the corresponding control release at the period of 45~64 min. High K^+ (56 mM)-evoked CA release in the presence of SKF81297 (30 μM) and SCH23390 (3 μM) for 60 min was also not changed for 0-49 min, but was highly inhibited to 75% of the corresponding control release only at the last period of 60~64 min period in comparison to the control secretion (154±16 ng, 0~4 min) from 5 glands (Fig. 4, Lower).

As shown in Fig. 5 (Upper), DMPP-evoked CA release prior to the perfusion with SKF81297 and SCH23390 was 435±24 ng (0~8 min). The simultaneous perfusion of SKF81297 and SCH23390 for 60 min no longer inhibited DMPP-evoked CA release for the period of 0~28 min in the 5 experiments while later became depressed to 80~85% of the control release at the period of 40~64 min. Moreover, in the presence of SKF81297 (30 μM) and SCH23390 (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 became inhibited to 69% of the corresponding control release (166±16 ng, 0~4 min) only in the last period of 60~64 min period from 5 glands (Fig. 5, Lower).

As shown in Fig. 6, the simultaneous perfusion of SKF81297 (30 μM) and SCH23390 (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, however was later depressed to 69% and 72% of the control release at the last period of 60~64 min in comparison to their corresponding control responses (166±16 ng/ 0~4 min and 156±12 ng/ 0~4 min, respectively).

DISCUSSION

These results described here in suggest that SKF81297 and SCH23390 inhibit and enhance the CA secretion from

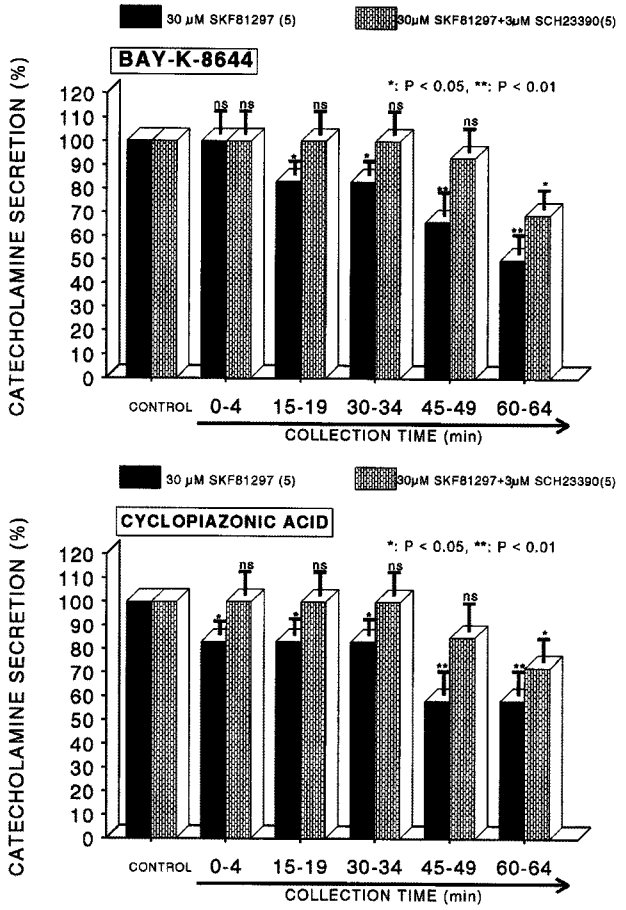

Fig. 6. Effects of SKF81297 plus SCH23390 on catecholamine release evoked by Bay-K-8644 (Upper) and cyclopiazonic acid (Lower) from the rat adrenal glands. Bay-K-8644 (10^{-5} M) and cyclopiazonic acid (10^{-5} M) were perfused into an adrenal vein for 4 min at 15 min intervals "BEFORE (CONTROL)" and "AFTER" preloading simultaneously with 30 μM SKF81297+3 μM SCH23390 for 60 min, respectively. Other legends are the same as in Fig. 1. *: $p<0.05$, **: $p<0.01$. ns: Statistically not significant.

the rat adrenal medulla, evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization, respectively. This inhibitory of SKF81297 seems to be mediated by stimulation of dopaminergic D_1 receptors located on the rat adrenomedullary chromaffin cells, while the facilitatory effect of SCH23390 is due to blockade of dopaminergic D_1 receptors, which are relevant to extra- and intracellular calcium mobilization. Therefore, the presence of the dopaminergic D_1 receptors may be involved in regulation of CA release in the rat adrenal medulla.

In support of these experimental results, Artalejo and his co-workers (1990) earlier showed specific binding of the rhodamine conjugate of the D_1 antagonist SCH-23390 to almost all the cells in the chromaffin cell culture. Because SCH-23390 binds with 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 on the cells are labeled. These observations suggest that D_5 receptors on the cells are responsible for inhibition of secretion by D_1-selective agonists. However, these D_5 receptors did not appear to be linked to adenylyl cyclase, and the stimulation of adenylyl cyclase is so weak that it was undetectable in the assay (Dahmer and Senogles, 1996). There are reports to suggest that there are 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 are in accordance with 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) 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 al, 1991); however, such a function for members of the D_1 family of dopamine receptors remains controversial.

The present results are consistent with those obtained previously. In the present work, pretreatment with SCH23390 moderately suppressed the SKF81297-induced inhibition of CA secretory responses evoked by ACh, high K^+, and DMPP. 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 rat. Furthermore, it is underscored by the finding that bilateral infusion of the D_1 receptor agonist, SKF81297, into rat prefrontal cortex (PFC) produced a dose-related impairment of spatial working memory that was reversed by D_1 antagonist pretreatment (Zahrt et al, 1996). Electrophysiological studies with awake, active monkeys also showed that iontophoresis of low concentrations of D_1 antagonists enhances memory-related neuronal firing (Williams and Goldman-Rakic, 1995). Infusions of the selective D_1 receptor antagonists, SCH23390 or SCH39166, into the the prefrontal cortex (PFC) of monkeys (Sawaguchi and Goldman-Rakic, 1991) or rats (Seamans et al, 1995) impaired spatial working memory performance, without altering performance of the control task with identical motor and motivational demands but little mnemonic component (Sawaguchi and Goldman-Rakic, 1991). In terms of these findings, it is quite possible 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 of arterial blood pressure was more important than in normal dogs (Damase-Michel et al, 1995). Heart rate was unchanged. In these animals, 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.

The nicotinic receptor is a neurotransmitter-gated cation-conducting ion channel that is opened by binding of agonists such as ACh and DMPP (McGehee and Role, 1995). The opening of this channel triggers Ca^{2+} uptake and secretion of CA from chromaffin cells (Wada et al, 1985b). To determine if the inhibition of DMPP-stimulated secretion by dopaminergic D_1 agonist was due to an effect on the activity of the nicotinic receptor, the effect of SKF81297 on

DMPP-stimulated CA secretion was examined. As shown in Fig. 5, treatment with SKF81297, a D_1-selective agonist, greatly inhibited DMPP-evoked CA secretion, reducing by 42% of the control release. The present data are very similar to the result that C1-APB, a D_1-selective agonist, inhibited DMPP-stimulated Na^+ uptake in bovine chromaffin cells (Dahmer and Senogles, 1996). Previous studies demonstrated that both D_1- and D_2-selective dopamine receptor agonists inhibit CA secretion and Ca^{2+} uptake, in bovine adrenal chromaffin cells, stimulated by the nicotinic ACh receptor agonist DMPP (Dahmer and Senogles, 1996). Both D_1- and D_2-selective agonists have also been found to inhibit CA release stimulated by veratridine, an agent that opens voltage-sensitive Na^+ channels (Dahmer and Senogles, 1996). It is, therefore, likely that SKF81297 can activate a signal transduction pathway, thus altering the activity of both nicotinic receptors and voltage-sensitive Na^+ channels. There have been reports that D_1-like dopamine receptors are linked to phosphoinositide metabolism (Felder et al, 1989; Andersen et al, 1990; Undie and Friedman, 1990). Activation of such a pathway could result in elevated levels of Ca^{2+}, diacylglycerol, and inositol bisphosphate in the cells. Consequently Ca^{2+}-dependent and protein kinase C-dependent pathways may be activated. Protein kinase C has been reported to attenuate the activity of both nicotinic receptors (Swope et al, 1992) and voltage-sensitive Na^+ channels (Catterall, 1992), and activation of D_1 receptors on chromaffin cells facilitates Ca^{2+} channels on the cells in a cAMP-dependent manner (Artalejo et al, 1990). However, Dahmer and Senogles (1996) could find no evidence of message for D_1 dopamine receptors in chromaffin cells by either PCR analysis or Northern blot analysis of RNA. Therefore, it appears that the facilitation of Ca^{2+} channels in these cells by SKF-38393 is due to activation of D_5 receptors, or that D_1 receptors must be present on only a subpopulation of the cells. In the present study, SKF81297 inhibited the CA secretory responses by high potassium as well as by Bay-K-8644, an activator of L-type Ca^{2+} channels, which facilitates the influx of Ca^{2+} into the cells. The observation that D_1-selective agonists inhibited the CA secretion evoked by Bay-K-8644 was surprising, since Artalejo et al (1990) reported that D_1-selective agonists facilitate Ca^{2+} current in bovine chromaffin cells. Although Ca^{2+} uptake measurements are clearly not the same as measuring Ca^{2+} channel activity, it is difficult to reconcile with data which indicate that D_1-selective agonists can inhibit Ca^{2+} uptake and facilitate Ca^{2+} channel activity. Again, one possible explanation is that only a subpopulation of chromaffin cells responds to dopamine agonists by using the facilitation channels.

It is unclear how activation of dopamine receptors results in the inhibition of secretion seen in these cells. The simplest interpretation is that the decrease of Ca^{2+} uptake by D_1-selective agonist is responsible for the observed inhibition of the CA secretion. However, such an interpretation appears to be too simple for the complexity of the relationship between the CA secretion and intracellular free Ca^{2+} levels. Both the intracellular location of the Ca^{2+} level increase (Cheek, 1989; Ghosh and Greenberg, 1995) and the magnitude of the Ca^{2+} level increase (Holz et al, 1982) can affect the relationship between intracellular free Ca^{2+} levels and secretion. Holz et al (1982) reported that when Ca^{2+} uptake was large, changes in Ca^{2+} uptake resulted in less than proportional changes in CA secretion. Consequently, although the decrease of Ca^{2+} uptake (influx) into the adrenal chromaffin cells may explain the decrease by SKF81297 in CA secretion, it is still unclear whether this is the only or even most important factor contributing to the inhibition of CA secretion by dopaminergic D_1 agonists. neverttheless, based on the results obtained in the present study, the voltage-sensitive calcium channel located on chromaffin cell membrane of the rat adrenal medulla could appears to be the target site for dopaminergic D_1-receptor-mediated inhibition of CA secretion.

In the present study, SKF81297 also inhibited the CA secretory responses evoked by cyclopiazonic acid, which is known to be a highly selective inhibitor of Ca^{2+}-ATPase in skeletal muscle sarcoplasmic reticulum (Geoger & Riley, 1989; Siedler et al, 1989). Therefore, the inhibitory effect of SKF81297 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 indicates that the activation of dopaminergic D_1-receptors has 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 time- and concentration-dependently produced the inhibition of CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests a new concept that SKF81297 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 support of this finding, it has been shown that cyclopiazonic acid penetrates easily into the cytoplasm through the plasma membrane and reduces Ca^{2+}-ATPase activity in sarcoplasmic/endoplasmic reticulum, resulting in an increase of subsequent Ca^{2+} release from those storage sites, thereby increasing Ca^{2+}-dependent K^+-current (Suzuki et al, 1992). Moreover, in bovine adrenal chromaffin cells, stimulation of muscarinic ACh receptors has also been proposed to cause activation of phosphoinositide metabolism, resulting in the formation of inositol 1,4,5-trisphosphate, which induces the mobilization of Ca^{2+} from the intracellular pools (Cheek et al, 1989; Challiss et al, 1991). However, in the present study, it is uncertain whether the inhibitory effect of SKF81297 on Ca^{2+} movement from intracellular pools was due to their direct effect on the PI response or an indirect effect.

Uceda and his coworkers (1992) reported that intracellular Ca^{2+}-dependent K^+ channels, probably of the small-conductance type (SK), seem to be involved in the modulation of muscarinic-evoked CA release responses in cat adrenal chromaffin cells. However, in the present study, the fact that McN-A-343-evoked CA secretion was depressed by pretreatment with SKF81297 appears to be consistent with these previous results. Furthermore, in the absence of extracellular Ca^{2+}, methacholine still evoked a transient Ca^{2+} rise that declined quickly to basal levels, suggesting that the release of Ca^{2+} from an intracellular pool is likely associated with the smooth endoplasmic reticulum in cat chromaffin cells (Uceda et al, 1992). In line with this observation are the facts that muscarinic stimulation of bovine chromaffin cells increases the formation of inositol trisphosphate (Forsberg et al, 1986), and that inositol trisphosphate mobilizes Ca^{2+} in permeabilized cells (Fohr et al, 1991). A similar rise of intracellular Ca^{2+} by muscarinic stimulation, even in the absence of extracellular Ca^{2+}, has been demonstrated in bovine chromaffin cell suspensions (Kim and Westhead, 1989) and in cat chromaffin cells

(Sorimach et al, 1992). Based on these previous results, the present finding suggests that the inhibitory dopaminergic D_1-receptors may be involved in regulating CA secretion evoked by muscarinic M_1-receptor stimulation, in the rat adrenal medullary chromaffin cells.

On the other hand, Collet and Story (1982a) found that dopamine inhibited the electrically evoked release of [^{3}H] NE from isolated perfused rabbit adrenal glands. This inhibition could completely be reversed by the dopamine D_2 selective antagonist, metoclopramide. It has been known that metoclopramide enhances the CA secretion in the perfused rat adrenal gland (Lim et al, 1989). Moreover, apomorphine dose-dependently inhibits the CA secretion induced by cholinergic receptor stimulation or membrane depolarization, from the isolated perfused rat adrenal gland, and this inhibition is attenuated by pretreatment with metoclopramide (Lim et al, 1994). Thus, the inhibition of CA secretory responses through D_2 dopaminergic activation is supported by several previous studies (Gonzalez et al, 1986; Lyon et al, 1987; Quick et al, 1987; Damase-Michel et al, 1999). This inhibitory D_2 dopaminergic effect has also been shown not to interact with D_1 receptors as described previously (Bigornia et al, 1988; 1990). These dopaminergic inhibitory effects in other systems are also 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) demonstrated that, in the same preparation of adrenomedullary samples in which significant numbers of D_2 receptors are found, there is no statistically significant specific binding of the D_1 receptor ligand, [^{3}H] SCH 23390. Moreover, 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). Based on these findings together with results the previously obtained from the rat adrenal medulla (Lim et al, 1994), it is clear that the dopaminergic D_2 receptors are involved in the regulation of CA secretion from the rat adrenomeduullary chromaffin cells. In contrast with the present results, Huettl and his colleagues (1991) concluded that pergolide and apomorphine inhibit CA release from bovine chromaffin cells in a non-receptor-mediated manner, 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 been shown that stimulation of dopaminergic D_1-receptors facilitates 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 facilitation of Ca^{2+} channels by dopamine may form the basis of a positive feedback loop mechanism that augments CA secretion. In anesthetized dogs, both quinpirole and apomorphine, selective D_2 dopaminergic agonists, failed to modify the release of EP and NE from the adrenal medulla, regardless of 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).

Taken together, the present results suggest that SKF81297 and SCH23390 inhibit and enhance the CA secretion from the rat adrenal medulla, evoked by cholinergic stimulation (both nicotininc and muscarinic receptors) and membrane depolarization, respectively. This inhibitory of SKF81297 seems to be mediated by stimulation of dopaminergic D_1 receptors located on the rat adrenomedullary chromaffin cells, whereas the facilitatory effect of SCH23390 is probably due to the blockade of dopaminergic D_1 receptors, which are relevant to extra- and intracellular calcium mobilization. Therefore, the presence of the dopaminergic D_1 receptors is likely involved in regulation of CA release in the rat adrenal medulla.

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