Arch Pharm Res Vol 25, No 4, 511-521, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Inhibitory Mechanism of Bromocriptine on Catecholamine Release Evoked by Cholinergic Stimulation and Membrane Depolarization from the Rat Adrenal Medulla

Dong-Yoon Lim, Yong-Gyoon Lee, Il-Hwan Kim

Department of Pharmacology, College of Medicine, Chosun University, Kwangju 501-759, KOREA

(Received April 1, 2002)

The purpose of this study was to determine whether bromocriptine affects the catecholamines (CA) secretion evoked in isolated perfused rat adrenal glands, by cholinergic stimulation, membrane depolarization and calcium mobilization, and to establish the mechanism of its action. The perfusion of bromocriptine (1~10 μM) into an adrenal vein, for 60 min, produced relatively dose-dependent inhibition in the secretion of catecholamines (CA) evoked by acetylcholine (ACh, 5.32 mM), DMPP (100 μM for 2 min), McN-A-343 (100 μM for 2 min), cyclopiazonic acid (CPA, 10 μM for 4 min) and Bay-K-8644 (10 μM for 4 min). High K^+ (56 mM)-evoked CA release was also inhibited, although not in a dose-dependent fashion. Also, in the presence of apomorphine (100 μM), which is also known to be a selective D_2-agonist, the CA secretory responses evoked by ACh, high potassium, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid were also significantly depressed. However, in adrenal glands preloaded with bromocriptine (3 μM) in the presence of metoclopramide (15 μM), a selective D_2-antagonist, the CA secretory responses evoked by ACh, high potassium, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid considerably recovered as compared to that of bromocriptine only. Taken together, these results suggest that bromocriptine can inhibit the CA secretion evoked by stimulation of cholinergic receptors, as well as by membrane depolarization, in the perfused rat adrenal medulla. It is thought this inhibitory effect of bromocriptine may be mediated by inhibiting the influx of extracellular calcium and the release from intracellular calcium stores, through the activation of dopaminergic D_2-receptors located in the rat adrenomedullary chromaffin cells. Furthermore, these findings also suggest that the dopaminergic D_2-receptors may play an important role in regulating adrenomedullary CA secretion.

Key words: Bromocriptine, Adrenal medulla, Catecholamine release, Dopaminergic D_2-Receptors

INTRODUCTION

It has become clear through molecular cloning that the dopamine receptor family has at least five subtypes, D_1~D_5 (Sibley & Monsma, 1992; O'Dowd, 1993). Dopamine receptors are now classified into two families based on; similarities in sequences, pharmacology and signal transduction pathways: D_1-like receptors (D_1 and D_5 receptors) or D_2-like receptors (D_2, D_3, and D_4 receptors). Effects attributed to dopamine include: modulation of behavior, movement, nerve conduction, blood pressure and hormone release. Initially two types of dopamine receptors, D_1 and D_2, were identified (Kebabian & Caline, 1979). These two receptors can be distinguished by the selective binding of various dopamine agonists and antagonists, sensitivity to toxins and signal transduction pathways; D_1 dopamine receptors activate adenylyl cyclase, whereas D_2 receptors either inhibit adenylyl cyclase or have no effect (Kebabian *et al.*, 1986; Vallar & Meldolesi, 1989). The presence of dopamine receptors in adrenomedullary chromaffin cells has been widely reported. Dopamine agonists have been found to inhibit the output of epinephrine and norepinephrine from perfused cat adrenal glands (Artalejo *et al.*, 1985; Gonzalez *et al.*, 1986), and from chromaffin cells maintained in primary cultures (Bigornia *et al.*, 1988; Bigornia *et al.*, 1990). The fact that specific D_2-receptor

Correspondence to: Dong-Yoon Lim, Professor, Department of Pharmacology, College of Medicine, Chosun University, Kwangju 501-759, KOREA
E-mail: dylim@chosun.ac.kr

antagonists reverse the inhibitory effect of dopamine indicates this effect is mediated by a D_2 receptor in the adrenal medulla. Furthermore, the presence of D_2, but not D_1, dopamine receptors was demonstrated in ligand binding studies on chromaffin cell membrane suspensions (Gonzalez *et al.*, 1986; Lyon *et al.*, 1987; Quick *et al.*, 1987). Recently, it has also been reported that dopaminergic inhibition of catecholamine secretion from adrenal medulla of dogs is mediated by D2-like, but not D1-like, dopaminergic receptors (Damase-Michel *et al.*, 1999). At the time, these data were interpreted as evidence that D_2 dopamine receptors, on the cells, inhibited CA release. However, molecular cloning had not yet identified D_3, D_4 and D_5 receptors. In retrospect, previously published data would not distinguish the D_2 dopamine receptor from the D_3 or D_4 receptor. Consequently it remains unclear which members of the D_2 family of dopamine receptors are responsible for the inhibition of secretion from adrenomedullary chromaffin cells.

However, in contrast to these findings, Artalejo and his coworkers (1990) identified D_1 dopaminergic receptors on bovine chromaffin cells by fluorescence microscopy. In addition, 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 been also 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). It has been demonstrated that apomorphine causes dose-dependent inhibition of CA secretion by cholinergic receptor stimulation, and membrane depolarization from isolated perfused rat adrenal glands (Lim *et al.*, 1994). Thus, it is clear that there are still many controversial reports on the modulating effects of dopaminergic D receptors in the CA release from the adrenal medulla. Therefore, the present study attempted to examine the effects of bromocriptine, a selective agonist of dopaminergic D_2 receptors, on the secretion of CA evoked by stimulation of cholinergic receptor and membrane depolarization in isolated perfused rat adrenal glands and to establish the mechanism of its action.

MATERIALS AND METHODS

Experimental procedure

Male Sprague-Dawley rats, weighing 180 to 300g, were intraperitoneally anesthetized with thiopental sodium (40 mg/kg). The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland, and surrounding area, was exposed by three hook retractors. The stomach, intestine and a portion of the liver were not removed, but pushed over to the right side, and covered by saline-soaked gauge pads. The 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 the vena cava to prevent blood coagulation prior to ligating vessels and cannulations. A small slit was made into the adrenal cortex, just opposite the entrance of the adrenal vein. Perfusion of the gland was started, making sure no leakage was present, and the perfusion fluid escaped only from the slit made in the adrenal cortex. Then the adrenal gland was carefully removed from the animal, along with ligated blood vessels and the cannula, and placed on a platform of a leucite chamber, which was continuously circulated with water heated at 37 ± 1°C.

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic WIZ pump (ISCO Inc., St. Lincoln, NE, U.S.A.) at a rate of 0.33 ml/min. The perfusion was carried out with Krebs-bicarbonate solution composed of the following (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. To prevent oxidation of catecholamines, the solution also contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) The solution was constantly bubbled with 95% O_2 + 5% CO_2 with the final pH of the solution being maintained at 7.4~7.5.

Drug administration

The perfusions of DMPP (10^{-4}M) and McN-A-343 (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 0.05ml, were made into the perfusion stream via a three-way stopcock, respectively. 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 upon administration of the above drugs, the secretory responses to ACh, KCl, McN-A-343, Bay-K-8644 and cyclopiazonic acid returned to preinjection levels in about 4 min, but that of DMPP in 8 min.

Collection of perfusate

Prior to stimulation with various secretagogues, the perfusate was collected for 4 min to determine the spontaneous secretion of CA, the background sample, after which the collection was continued, in another tube,

as soon as the perfusion medium, containing the stimulatory agent, reached the adrenal gland. Stimulated samples was collected for 4 to 8 min. The amount secreted in the background sample was subtracted from that in the stimulated sample, and the net secretion value of CA obtained, which as shown in the figures.

To study the effect of the bromocriptine on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing bromocriptine for 60 min. Then the perfusate was collected for the specified period (background sample), followed by collection of the medium containing the stimulating agent.The perfusate samples were collected for the same period as that of the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

The CA content of the perfusate was measured directly by the fluorometric method of Anton and Sayre (Anton and Sayre, 1962), but without the intermediate purification alumina, for the reasons described earlier (Wakade, 1981), using a fluorospectrophotometer (Kontron Co., Milan, Italy).

A 0.2 ml volume of the perfusate was used in the reaction. The CA content in the perfusate, of stimulated glands by secretagogues, used in this work was high enough to obtain readings several folds greater than those of the control samples (unstimulated). The sample blanks were those perfusates of stimulated and non-stimulated samples with the lowest readings. The content of the CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statistical analysis

The statistical significance between groups was analyzed *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 their standard errors (S.E.M.). The statistical analyses of the experimental results were made by the computer program described by Tallarida and Murray (1987).

Drugs and their sources

The following drugs were used: bromocriptine mesylate, apomorphine hydrochloride, acetylcholine chloride, 1.1-dimethyl-4-phenyl piperazinium iodide (DMPP), norepinephrine bitartrate, metoclopramide hydrochloride, methyl-1, 4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl) -pyridine-5-carboxylate (BAY-K-8644) (Sigma Chemical Co. U.S.A.) and cyclopiazonic acid, (3-(m-chloro-phenyl-carbamoyl-oxy)-2butynyl 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, with the exception of the Bay-K-8644,

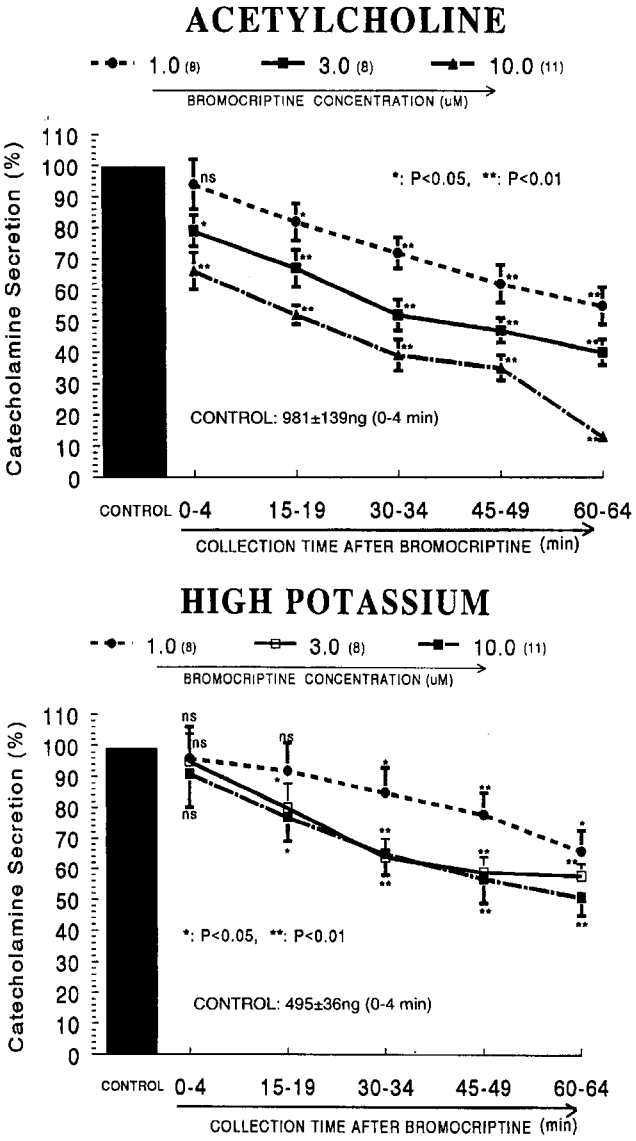

Fig. 1. Effects of bromocriptine on the secretory responses of catecholamines (CA) evoked by acetylcholine (left), and high potassium (right), from the isolated perfused rat adrenal glands. CA secretion, by a single injection of ACh (5.32×10^{-3}M) and excess K^+(5.6×10^{-2}M), was induced "BEFORE" and "AFTER" preloading with bromocriptine (10^{-6}~10^{-5}M) for 60 min. Numbers in the parenthesis indicate the number of experimental rat adrenal glands. Vertical bars represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from adrenal gland (% of the control). Abscissa: collection time (min). Statistical difference was obtained by comparing the corresponding "BEFORE" (control) with each period "AFTER" the initiation of bromocriptine perfusion. Perfusates were collected for 4 minutes at 15 min intervals. $P < 0.05$, ": $P < 0.01$, ns: not statistically significant.

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

After one hour of the initial perfusion with oxygenated Krebs-bicarbonate solution, the spontaneous CA released from the isolated perfused rat adrenal glands was 24 ± 3 ng/2 min (n=12). It had been already found that in normotensive subjects, bromocriptine lowers both levels of plasma and cerebrospinal fluid norepinephrine by 50%, lowers blood pressure moderately in standing subjects and slightly in recumbent subjects (Ziegler *et al.*, 1979; Van Loon *et al.*, 1979). Therefore, it was decided initially to examine the effects of bromocriptine on cholinergic receptor stimulation-, as well as membrane depolarization-mediated, CA secretion from perfused rat adrenal glands. Secretagogues were given at 15 min-intervals. The bromocriptine was present for 60 min, including periodic stimulation with each secretagogue.

In the present study, the bromocriptine itself was found not to produce any effect on the spontaneous CA secretion (data not shown). When injected in a volume of 0.05ml into the perfusion stream, ACh (5.32×10^{-3}M)-evoked CA secretion of 981 ± 139 ng (0-4 min). However, in the presence of bromocriptine (1~10 μM), the ACh-evoked CA secretion was decreased dose-dependently by 13% of that of the control (100%), as shown in Fig. 1.

It has been found that direct membrane-depolarizing agent, like high potassium, sharply stimulates CA secretion. In this work, high K^+ (5.6×10^{-2}M)-evoked CA secretion, following the pretreatment with bromocriptine, was initially unaffected, but was later followed by inhibition. In the presence of bromocriptine (1~10 μM), the high K^+-evoked CA release was gradually reduced by 51% of its corresponding control secretion (495 ± 36ng/0-4 min) (Fig. 1). Strangely, high K^+-evoked CA secretion, following bromocriptine pretreatment, did not show a dependent inhibition, but the reason for this was not elucidated.

When the DMPP (10^{-4} M for 2 min), a selective nicotinic receptor agonist in autonomic sympathetic ganglia, was perfused through the rat adrenal gland, a sharp and rapid increase in CA secretion was evoked. As shown in Fig. 2, the DMPP-evoked CA release, prior to perfusion with the bromocriptine, was 1223 ± 223ng (0-8 min), while in the presence of the bromocriptine (1~10 μM), they were time-

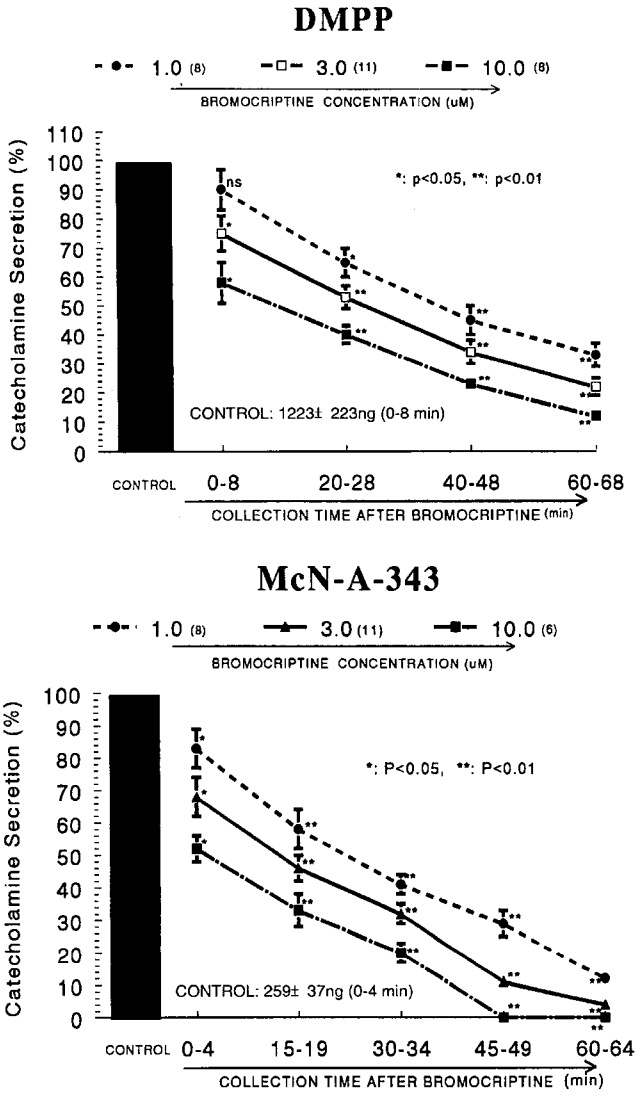

Fig. 2. Effects of bromocriptine on the secretory responses of catecholamines (CA) evoked by DMPP (left), and McN-A343 (right), from the isolated perfused rat adrenal glands. CA secretion by perfusion of DMPP (10^{-4}M), and McN-A-343 (10^{-4}M), was induced before (CONTROL) and after perfusion with bromocriptine (10^{-6}~10^{-5}M) for 60 min. DMPP-induced perfusates were collected for 8 minutes at 20 min interval, and McN-A-343-induced perfusates for 4 minutes at 15 min interval. Other legends are the same as in Fig. 1. *: P <0.05, **: P < 0.01, ns: not statistically significant.

and dose-dependently reduced by 12% from that of the control (100%), respectively. As illustrated in Fig. 2, the 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 2 min caused an increased in the CA secretion to 259 ± 37ng (0-4 min). However, the McN-A-343-evoked CA secretion in the presence of bromocriptine (1~10 μM) was greatly inhibited by 76~0% from that of the control (100%), respectively. These results are consistent with those of previous studies on

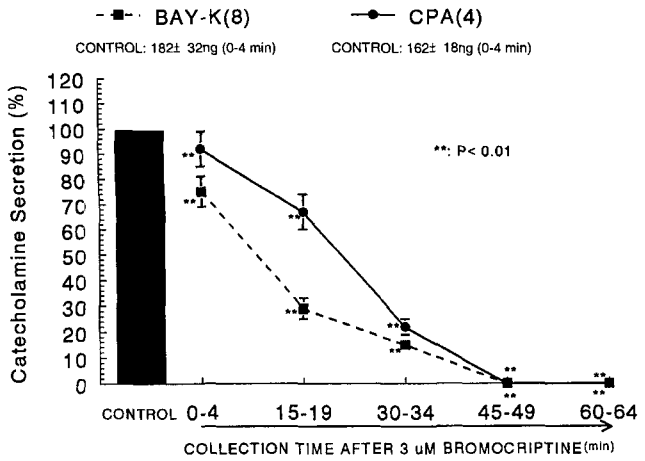

Fig. 3. Effects of bromocriptine on secretory responses of catecholamines (CA) evoked by Bay-K-8644 (left), and cyclopiazonic acid (right), from the isolated perfused rat adrenal glands. CA secretion by perfusion of Bay-K-8644 (BAY-K, 10^{-5} M) and cyclopiazonic acid (CPA 10^{-5}M) for 4 min was induced before and after perfusion with 3μM bromocriptine for 60 min. Bay-K-8644- and CPA-induced perfusates were collected for 4 minutes at 15 min-intervals. Other legends are the same as in Fig. 1. **: P < 0.01.

perfused cat adrenal glands (Artalejo *et al.*, 1985; Gonzalez *et al.*, 1986), cultured bovine chromaffin cells (Bigornia *et al.*, 1988; Bigornia *et al.*, 1990) and adrenal medulla in dogs (Damase-Michel *et al.*, 1999).

Effect of bromocriptine on 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 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 CA release (Lim *et al.*, 1992). Therefore, it was of interest to examine the effects of bromocriptine (3 μM) on the Bay-K-8644-evoked CA secretion from the isolated perfused rat adrenal glands. Fig. 3 shows the inhibitory effect of bromocriptine (3 μM) on the Bay-K-8644-evoked CA secretory responses. In the absence of bromocriptine, Bay-K-8644 (10^{-5}M) introduced into the perfusion stream evoked CA secretion of 182 ± 32 ng (0-4 min) in 8 rat adrenal glands. However, in the presence of bromocriptine (3 μM), the Bay-K-8644-stimulated CA secretion was time-dependently inhibited by 75~0% to that of the corresponding control release (100%).

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 may be an extremely valuable pharmacological tool for investigating intracellular Ca^{2+} mobilization and ionic current regulated by intracellular calcium (Suzuki *et al.*, 1992). As shown in Fig. 3, in the presence of 3 μM bromocriptine, the cyclopiazonic acid (10^{-5}M)-evoked CA secretion was largely attenuated by

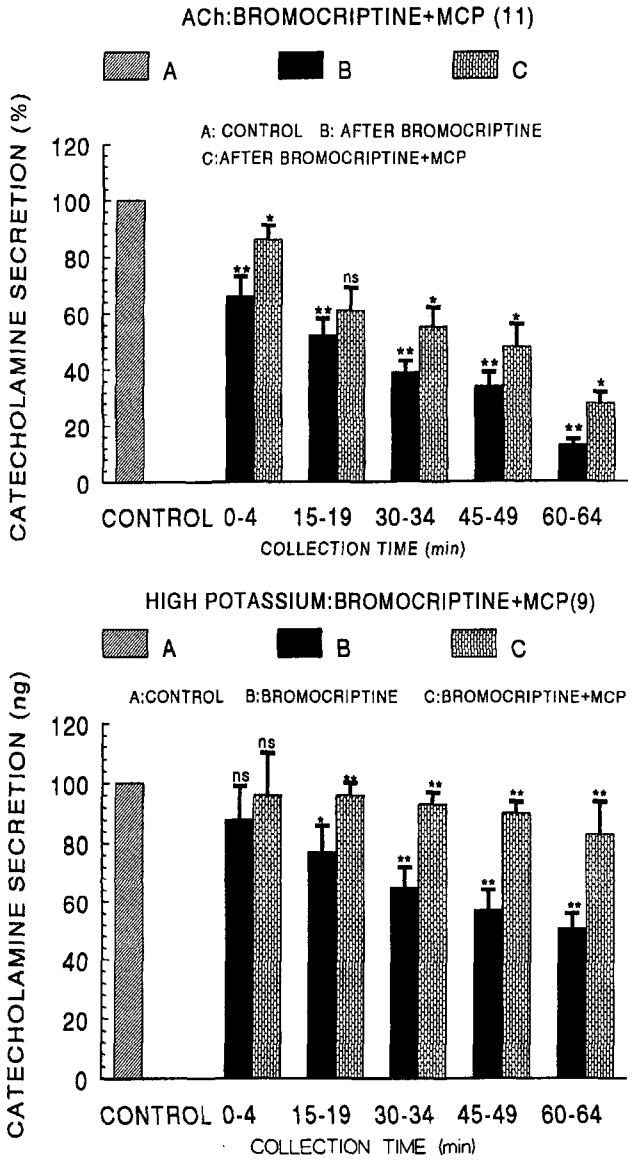

Fig. 4. Effects of bromocriptine plus metoclopramide on CA release evoked by acetylcholine (upper) and high potassium (lower) from the isolated perfused rat adrenal glands. CA secretion by a single injection of Ach (5.32×10^{-3}M) and high K^+ (5.6×10^{-2}M) was induced before (CONTROL, A) and after preloading with 3×10^{-6}M bromocriptine only (B) or 3×10^{-6}M bromocriptine + 1.5×10^{-5}M metoclopramide (C) for 60 min. Ordinate: the amounts of CA secreted from the adrenal gland (%). Abscissa: Collection time of perfusate (min). Statistical difference was obtained by comparing "A" with "B", and by comparing "B" with "C". MCP: Metoclopramide. *: P <0.05, **: P < 0.01, *ns*: not statistically significant.

90~0% to that of thecontrol (162 ± 18 ng/0-4 min, 100%) from 8 glands.

Effects of bromocriptine plus metoclopramide on CA release evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid from the perfused rat adrenal glands

In this, it was found that bromocriptine, time- and dose-dependently, inhibited the CA secretion evoked by cholinergic receptor stimulation and membrane depolarization of the rat adrenal glands, as shown in Fig. 1~3. Therefore, it was of much interest to examine the effects of bromocriptine, in the presence of metoclopramide, on CA secretion evoked by the cholinergic receptor stimulation and membrane depolarization of the isolated rat adrenal glands. It has been shown that the bromocriptine-induced hypotensive responses are blocked by various dopaminergic receptors antagonists, such as pimozide, metoclopramide, sulpiride or domperidone (Roquebert *et al.*, 1990; Luchsinger *et al.*, 1995; Blanco *et al.*, 1997).

When introduced into the adrenal vein in 11 rat adrenal glands, the ACh (5.32 mM)-evoked CA releasing resaponses, in the presence of 3 μM bromocriptine with 15 μM metoclopramide for 60 min, were recovered considerably to 86~28% of their control secretion compared to their secretory responses of 66~13% of their controls in the presence of 3 μM bromocriptine only (Fig. 4). Conversely, treatment with bromocriptine plus metoclopramide failed to alter the basal CA secretory response (data not shown).

As depicted in Fig. 4, high potassium (56 mM)-evoked CA secretory responses were also reversed considerably to 96~83% of their control secretion, compared to their secretory responses of 88~51% of their controls in the presence of 3 μM bromocriptine only. The neuronal nicotinic agonist, DMPP (10^{-5} μM)-evoked CA releasing responses, in the presence of 3 μM bromocriptine with 15 μM metoclopramide for 60 min, were recovered to 90~37% of their control secretion (100%), compared to their secretory responses of 82~24% of their controls in the presence of 3 μM bromocriptine only (Fig. 5). The muscarinic M_1-receptor agonist, McN-A-343 (10^{-5} μM), evoked CA secretions, in the presence of bromocriptine plus 15 μM metoclopramide for 60 min, were also recovered considerably to 85~18% of their corresponding control responses (100%), compared to the secretory responses of 76~4% of the control in the presence of 3 μM bromocriptine only (Fig. 5).

The Bay-K-8644 (10 μM)-evoked CA secretory responses, in the presence of bromocriptine plus 15 μM metoclopramide for 60 min, returned to 85~0% of their corresponding control responses (100%), compared to

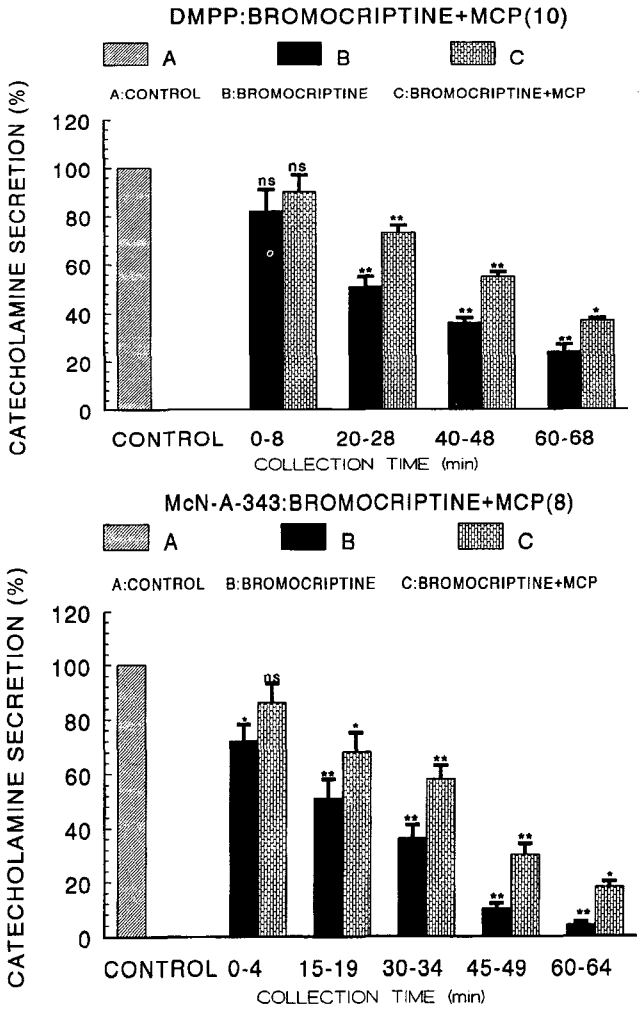

Fig. 5. Effects of bromocriptine plus metoclopramide on CA release evoked by DMPP (upper) and McN-A-343 (lower) from the isolated perfused rat adrenal glands. CA secretion by the perfusion of DMPP (10^{-4}M) and McN-A-343 (10^{-4}M) for 2 min was induced before (A) and after preloading with 3×10^{-6}M bromocriptine only (B) or 3×10^{-6}M bromocriptine + 1.5×10^{-5}M metoclopramide (C) for 60 min. Other legends are the same as in Fig. 4. *: $P < 0.05$, **: $P < 0.01$, ns: not statistically significant.

their secretory responses of 88~0% of the control in the presence of 3 μM bromocriptine only (Fig. 6). As shown in Fig. 6, the cyclopiazonic acid (10 μM)-evoked CA secretory responses were markedly reversed to 85~0% of their control secretion (100%), compared to their secretory responses of 69~0% of their controls in the presence of 3 μM bromocriptine only.

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

From the experimental results, as shown in Fig. 1~6, bromocriptine showed time- and dose-dependent inhibition of the CA secretory responses. It has already

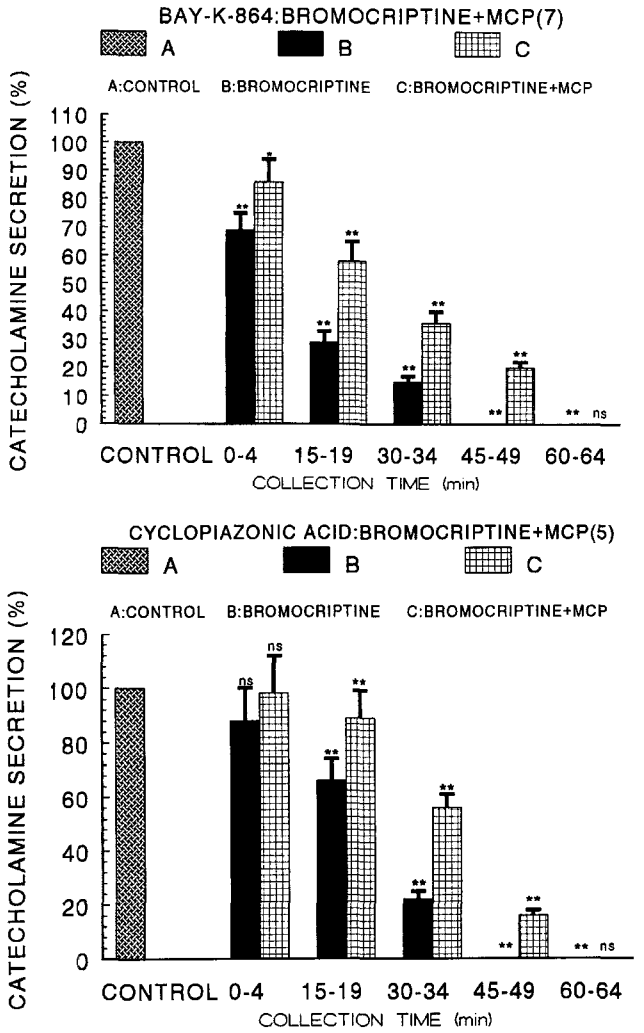

Fig. 6. Effects of bromocriptine plus metoclopramide on CA release evoked by Bay-K-8644 (upper) and cyclopiazonic acid (lower) from the isolated perfused rat adrenal glands. CA secretion by the perfusion of Bay-K-8644 (10^{-5}M) and cyclopiazonic acid (10^{-5}M) for 4 min was induced before (A) and after preloading with 3×10^{-6}M bromocriptine only B) or 3×10^{-6}M bromocriptine + 1.5×10^{-5}M metoclopramide (C) for 60 min. Other legends are the same as in Fig. 4. *: $P < 0.05$, **: $P < 0.01$, ns: not statistically significant.

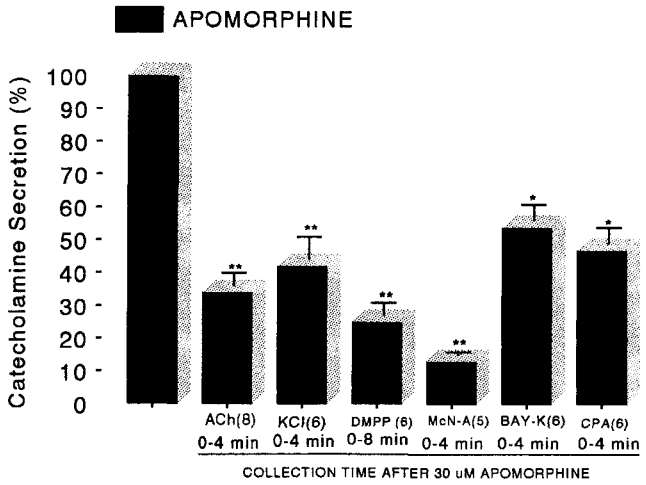

Fig. 7. Effects of apomorphine on CA release evoked by ACh, excess K^+, DMPP-, McN-A-343, Bay-K-8644 and cyclopiazonic acid. CA secretion evoked by ACh (5.32×10^{-3}M), excess K^+ (5.6×10^{-2}M), DMPP (10^{-4}M), McN-A-343 (10^{-4}M), Bay-K-8644 (10^{-5}M) and cyclopiazonic acid (10^{-5}M) were induced before and after preloading with apomorphine (3×10^{-5}M) for 20 min. Other legends are the same as in Fig. 1, 2 and 3*: $P < 0.05$, **: $P < 0.01$.

been reported that apomorphine, an agonist of dopaminergic D_2-receptor, also inhibits CA secretion evoked by cholinergic receptor stimulation and membrane depolarization in rat adrenal glands (Lim *et al.*, 1994). Therefore, it was of interest to examine effect of apomorphine on CA secretion evoked by; ACh, high K^+, DMPP and McN-A-343 from the isolated perfused rat adrenal glands.

The CA release evoked by ACh (5.32 mM) and high K^+ (56 mM), following pre-loading with 30 μM apomorphine for 20 min, were greatly reduced by 34% ($P < 0.01$, n=8) and 42% ($P < 0.01$, n=6), respectively, compared to the corresponding control secretions (100%), as shown Fig. 7.

The DMPP (100 μM)- and McN-A-343 (100 μM)-evoked CA releases, following preloading with 30 μM apomorphine for 20 min, were significantly reduced by 25% ($P < 0.05$, n=6) and 13% ($P < 0.01$, n=6), respectively, compared to their corresponding control responses (100%), as shown in Fig. 7. In the presence of 30 μM apomorphine, the cyclopiazonic acid (10 μM)-evoked CA secretory response was depressed by 47% ($P < 0.05$, n=6) of the corresponding control response (100%). As illustrated in Fig. 7, in the presence of 30 μM apomorphine, the Bay-K-8644-evoked CA secretion was also strikingly depressed by 54% ($P < 0.05$, n=6) of the corresponding control release (100%).

DISCUSSION

The results obtained in this study indicate that the dopaminergic D_2-receptor agonist bromocriptine greatly inhibits CA secretion evoked by ACh, high K^+, DMPP, McN-A-343, Bay-K-8644 and cyclopiazonic acid in isolated perfused rat adrenal glands. Bromocriptine itself, at the concentrations used, did not affect spontaneous CA release. In the presence of the dopamine receptor antagonist, metoclopramide, the bromocriptine-induced inhibitory effects of CA release, evoked by cholinergic receptor stimulation and membrane depolarization, were reversed considerably, compared to the results obtained from pretreatment with bromocriptine only. Therefore, it could be concluded that this inhibitory effect of

bromocriptine appears to be due to the inhibition of both extracellular calcium influx into the rat adrenal medullary chromaffin cells, and calcium release from the cytoplasmic calcium store, through the activation of inhibitory dopaminergic D_2-receptors.

In support of our experimental results, it has been reported that the presence of D_2 dopaminergic receptors, on adrenal chromaffin cells, can be demonstrated by radioligand binding methods (Gonzalez *et al.*, 1986; Lyon *et al.*, 1987; Quick *et al.*, 1987). These dopaminergic receptors, located on chromaffin cells, appear to function as an inhibitory modulator of adrenal CA secretion, as shown in the results obtained in cultured bovine adrenal chromaffin cells (Bigornia *et al.*, 1988; 1990) and in some studies with perfused cat adrenal glands (Artalejo *et al.*, 1985; Gonzalez *et al.*, 1986; Montastruc *et al.*, 1989). 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 the stimulation of the nicotinic cholinoceptor (Artalejo *et al.*, 1985). The fact that dopamine is released in measurable amounts, together with epinephrine and norepinephrine from perfused cat adrenal glands in response to nicotinic stimulation, favors a role for this dopaminergic receptor in modulating CA release from the chromaffin cells. Moreover, it has been also found that, as in cats, bovine adrenal glands contain dopaminergic receptors that modulate CA secretion evoked by the stimulation of nicotinic cholinergic receptors through the activation of inhibitory D_2 type receptors (Gonzalez *et al.*, 1986). A subcutaneous injection of apomorphine, in normotensive rats, has been found to produce a dose-dependent decrease in the 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 data obtained in the investigation of bovine adrenal chromaffin cells could support dopaminergic D_2-receptors appear to function as inhibitory modulators of adrenal CA secretion (Bigornia *et al.*, 1988; 1990). Furthermore, the inhibitory effects of apomorphine or dopamine on the nicotine-evoked CA secretion are antagonized, or reversed, by pretreatment with the dopaminergic D_2 antagonists, domperidore, sulpiride, haloperidol and metoclopramide (Collet & Story, 1982a; 1982b; Artalejo *et al.*, 1985; Montiel *et al.*, 1986; Montastruc *et al.*, 1989). These previous results are consistent with those obtained from our study. We found the pretreatment with metoclopramide reversed the bromocriptine-induced inhibition of the CA secretory process evoked by ACh, high K^+ and DMPP. This fact confirms that bromocriptine inhibits CA secretory responses evoked by nicotinic stimulation and 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 10^{-6} M dopamine inhibited the electrically evoked release of [^{3}H] norepinephrine in isolated perfused rabbit adrenal glands. This inhibition could be completely reversed by the dopamine D_2 selective antagonist metoclopramide (3×10^{-6}M). In previous experiments, it has been shown that metoclopramide evokes CA secretion in perfused rat adrenal glands (Lim *et al.*, 1989). Moreover, it has been demonstrated that apomorphine causes a dose-dependent inhibition of the CA secretion by cholinergic receptor stimulation and membrane depolarization from isolated perfused rat adrenal glands. These inhibitory effects appear to be exerted by inhibiting the influx of extracellular calcium into the rat adrenomedullary chromaffin cells through the activation of inhibitory dopaminergic receptors (Lim *et al.*, 1994).

The results from this work, where bromocriptine was found to cause inhibition of the CA secretory responses evoked by ACh, DMPP and excess K^+ through D_2 dopaminergic activation, were supported by several previous studies (Gonzalez *et al.*, 1986; Lyon *et al.*, 1987; Quick *et al.*, 1987; Damase-Michel *et al.*, 1999), although the effect of the D_1 receptors was not examined in this study. 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). Consistent with our results, the dopaminergic inhibitory effects in other systems have been found to be mediated, specifically, by the D_2-receptor subtype (Memo *et al.*, 1985; de Vlieger *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 was no statistically significant specific binding of the D_1 receptor ligand, [^{3}H] SCH 23390. More recently, it has been shown that dopaminergic inhibition of CA secretion, from adrenal medulla of conscious male beagle dogs, is mediated by the activation of D_2-like, but not D_1-like, dopaminergic receptors (Damase-Michel *et al.*, 1999).

The results of this work also illustrate that bromocriptine produces the inhibitory modulation of adrenal CA secretion, at least partly, by the inhibition of calcium channel currents through stimulation of the inhibitory dopaminergic D_2 receptors. Our finding, that bromocriptine greatly 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, demonstrates that the bromocriptine-induced inhibitory effect on CA release is due to the blockade of the voltage-sensitive calcium channels. 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 either nicotine (10 μM) or membrane depolarization, with an elevated K^+ level (60mM), 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 almost completely in agree with those found by ourselves.

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 dopaminergic D_2-receptor-mediated inhibition of CA secretion. This would also be consistent with the findings in several other systems. Moreover, it is well established that dopamine inhibits prolactin secretion from anterior pituitary cells, and this appears to be mediated by a specific D_2-receptor (Caro *et al.*, 1978), and there is some evidence that Ca^{2+} may play a role in the dopamine-mediated inhibition of prolactin secretion (Schettini *et al.*, 1983; Merritt & Brown, 1984).

In the present study, bromocriptine was also found to inhibit 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, it is felt that the inhibitory effect of bromocriptine on the CA secretion, evoked by cholinergic stimulation and membrane-depolarization, may be associated with the mobilization of intracellular Ca^{2+} in the chromaffin cells. This would indicate that the activation of dopaminergic D_2-receptors has an inhibitory effect on the release of Ca^{2+} from the intracellular pools induced by the stimulation of muscarinic ACh receptors, which is weakly responsible for the secretion of CA. We have shown bromocriptine to time- and concentration-dependently produced the inhibition of the CA secretion evoked by McN-A-343, a selective muscarinic M_1-agonist. This fact suggests another new concept, where the bromocriptine can modulate the CA secretory process induced by the activation of muscarinic M_1- and neuronal nicotinic receptors in the rat adrenal medulla. To support this finding, it has been shown that cyclopiazonic acid easily penetrates the cytoplasm through the plasma membrane and reduces Ca^{2+}-ATPase activity in the sarcoplasmic/endoplasmic reticulum, resulting in an increase in the subsequent Ca^{2+} release from those storage sites, thereby increasing the 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 this study, it is uncertain whether the inhibitory effect of the bromocriptine on Ca^{2+} movement from intracellular pools is due to their direct effect on the PI response, or as an indirect effect from the result of the membrane hyperpolarization, which is induced by opening of K^+ channels.

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 bromocriptine appears to be consistent with the 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 the release of Ca^{2+} from an intracellular pool were likely to be associated with the smooth endoplasmic reticulum in cat chromaffin cells (Uceda *et al.*, 1992). In line with this observation, the muscarinic stimulation of bovine chromaffin cells increases the formation of inositol trisphosphate (Forsberg *et al.*, 1986), and inositol trisphosphate is known to mobilize Ca^{2+} in permeabilized cells (Fohr *et al.*, 1991). A similar rise of intracellular Ca^{2+}, caused by muscarinic stimulation even in the absence of extracellular Ca^{2+}, has been demonstrated in bovine chromaffin cell suspensions (Kim & Westhead, 1989) and cat chromaffin cells (Sorimach, Yamagami & Nishimura, 1992). Based on previous results, the finding presenting this work suggests that the inhibitory dopaminergic D_2-receptors may be involved in regulating the CA secretion evoked by the muscarinic M_1-receptor stimulation in rat adrenal medullary chromaffin cells.

In contrast with the present experimental results, Huettl and his colleagues (1991) concluded; pergolide and apomorphine act in a nonreceptor-mediated manner to inhibit CA release from bovine chromaffin cells, and functional dopaminergic D_2 receptors, of the classical type, do not exist on isolated bovine chromaffin cells. The inhibitory effect, of the selective dopaminergic D_2 agonists pergolide and 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 conclusion, collectively, the results from the present study demonstrate that bromocriptine can inhibit the CA secretion evoked by stimulation of cholinergic receptors, as well as by membrane depolarization, in the perfused rat adrenal medulla. It is felt that this inhibitory effect is mediated by the inhibition of both the influx of extracellular calcium, and its release from intracellular calcium stores,

through the activation of inhibitory dopaminergic D_2-receptors located in the rat adrenomedullary chromaffin cells. Furthermore, these findings also suggest that the dopaminergic D_2-receptors may play an important role in regulating adrenomedullary CA secretion.

AKNOWLEDGEMENTS

This study was supported partly by a research fund of Chosun University (2001).

REFERENCES

Anton, A.H., and Sayre, D.F., A study of the factors affecting the aluminum oxide trihydroxy indole procedure for the analysis of catecholamines. *J. Pharmacol. Exp. Ther.,* 138, 360-375 (1962).

Artalejo, A.R., Ariano, M.A., Perlman, R.L., and Fox, A.P., Activation of facilitation calcium channels in chromaffin cells by D_1 dopamine receptors through an AMP/protein kinase A-dependent mechanism. *Nature,* 348, 239-242 (1990).

Artalejo, A.R., Garcia, A.G., Montiel, C., and Sanchez-Garcia, P., A dopaminergic receptor modulates catecholamine release from the cat adrenal gland. *J. Physiol.,* 362, 359-368 (1985).

Bigornia, L., Allen, C.N., Jan, C.R., Lyon, R.A., Titeler, M., and Schneider, A.S., D_2 dopamine receptors modulate calcium channel currents and catecholamine secretion in bovine adrenal chromaffin cells. *J. Pharmacol. Expt. Ther.,* 252(2), 586-592 (1990).

Bigornia, L., Suozzo, M., Ryan, K.A., Napp, D., and Schneider, A.S., Dopamine receptors on adrenal chromaffin cells modulate calcium uptake and catecholamine release. *J. Neurochem.,* 51, 999-1006 (1988).

Blanco, M., Hurtado, N., Jelambi, I., Perez, G., Carrillo, M., Gomez, J., Bravo, C., Gomez, H., Collet, H., and Velasco, M., Dopaminergic influence on cardiovascular responses to exercise stress in hypertensive subjects. *Am. J. Ther.,* 4, 31-33 (1997).

Caron, M.G., Beaulieu, M., Raymond, V., Gagne, B., Drouin, J., Lefkowitz, R.J., and Labrie, F., Dopaminergic receptors in the anterior pituitary gland. *J. Biol. Chem.,* 253, 2244-2253 (1978).

Challiss, R.A.J., Jones, J.A., Owen, P.J., and Boarder, M.R., Changes in inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate mass accumulations in cultured adrenal chromaffin cells in response to bradykinin and histamine. *J. Neurochem.,* 56, 1083-1086 (1991).

Cheek, T.R., O'Sullivan, A.J., Moreton, R.B., Berridge, M.J., and Burgoyne, R.D., Spatial localization of the stimulus-induced rise in cytosolic Ca^{2+} in bovine adrenal chromaffin cells: Distinct nicotinic and muscarinic patterns. *FEBS. Lett.,* 247, 429-434 (1989).

Collet, A.R., and Story, D.F., Is catecholamine release from the rabbit adrenal gland subject to regulation through dopamine receptors or β-adrenoceptors? *Clin. Exp. Pharmacol. Physiol.,* 9, 436 (1982a).

Collet, A.R., and Story, D.F., Release of ^{3}H-adrenaline from an isolated intact preparation of the rabbit adrenal gland: No evidence for release modulatory α-adrenoceptors. *J. Auton. Pharmacol.,* 2, 25-34 (1982b).

Cooper, D.M.F., Bier-Laning, C.M., Halford, M.K., Ahlijanian, M.K., and Zahniser, N.R., Dopamine acting through D_2 receptors inhibits rat striatal adenylate cyclase by a GTP-dependent process. *Mol. Pharmacol.,* 29, 113-119 (1986).

Dahmer, M.K., and Senogles, S.E., Differential inhibition of secretagogue-stimulated sodium uptake in adrenal chromaffin cells by activation of D_4 and D_5 dopamine receptors. *J. Neurochem.,* 67, 1960-1964 (1996).

Damase-Michel, C., Montastruc, J.L., Geelen, G., Saint-Blanquat, G.D., and Tran, M.A., Effect of quinpirole a specific dopamine DA2 receptor agonist on the sympathoadrenal system in dogs. *J. Pharmacol. Expt. Ther.,* 252(2), 770-777 (1990).

Damase-Michell, C., Montastruc, J.L., and Tran, M.A., Dopaminergic inhibition of catecholamine secretion from adrenal medulla is mediated by D_2-like but not D_1-like dopamine receptors. *Clin. Expt. Pharmacol. Physiol.,* 26(suppl.), S67-S68 (1999).

De Vliefer, T.A., Lodder, J.C., Werkman, T.R., and Stoof, J.C., Dopamine receptor stimulation has multiple effects on ionic currents in neuroendocrine cells of the pond snail *Lymnaea stagnalis*. *(Abstr) Neuroscience Lett [Suppl].,* 22, S418 (1985).

Fohr, K.J., Ahnert-Hilger, G., Stecher, B., Scott, J., and Gratzl, M., GTP and Ca^{2+} modulate the inositol 1,4,5-trisphosphate-dependent Ca^{2+} release in streptolysin O-permeabilized bovine adrenal chromaffin cells. *J. Neurochem.,* 56, 665-670 (1991).

Forsberg, E.J., Rojas, E., and Pollard, H.P., Muscarinic receptor enhancement of nicotinic-induced catecholamine secretion may be mediated by phosphoinositide metabolism in bovine adrenal chromaffin cells. *J. Biol. Chem.,* 261, 4915-4920 (1986).

Garcia, A.G., Sala, F., Reig, J.A., Viniegra, S., Frias, J., Fonteriz, R., and Gandia, L., Dihydropyridine Bay-K-8644 activates chromaffin cell calcium channels. *Nature,* 309, 69-71 (1984).

Goerger, D.E., and Riley, R.T., Interaction of cyclopiazonic acid with rat skeletal muscle sarcoplasmic reticulum vesicles. Effect on Ca^{2+} binding and Ca^{2+} permeability. *Biochem. Pharmacol* ., 38, 3995-4003 (1989).

Gonzales, M.C., Artalejo, A.R., Montiel, C., Hervas, P.P., and Garcia, A.G., Characterization of a dopaminergic receptor that modulates adrenomedullary catecholamine release. *J. Neurochem.,* 47, 382-388 (1986).

Huettl, P., Gerhardt, G.A., Browning, M.D., and Masserano, J.M., Effects of dopamine receptor agonists and antagonists on catecholamine release in bovine chromaffin cells. *J. Pharmacol. Expt. Ther.,* 257(2), 567-574 (1991).

Kebabian, J. W., and Calne, D. B., Multiple receptors for dopamine. *Nature,* 277(5692), 93-96 (1979).

Kebabian. J. W., Augi, T., van Oene. J. C., Shigematsu. K., and Saavedra, J. M., The dopamine receptor: New perspectives. *Trends. Pharmacol. Sci.,* 7, 96-99 (1986).

Kim, K.T., and Weathead, E.W., Cellular responses of Ca^{2+} from extracellular and intracellular sources are different as shown by simultaneous measurements of cytosolic Ca^{2+} and secretion from bovine chromaffin cells. *Proc. Natl. Acad .Sci. USA., 86,* 9881-9885 (1989).

Lim, D.Y., Kim, C.D., and Ahn, K.W., Influence of TMB-8 on secretion of catecholamines from the perfused rat adrenal glands. *Arch. Pharm. Res.,* 15(2), 115-125 (1992).

Lim, D.Y., Kim, K. H., Choi, C.H., Yoo, H.J., Choi, D.J., and Lee, E.H., Studies on secretion of catecholamines evoked by metoclopramide of the rat adrenal gland. *Korean J. Pharmacol.,* 25(1), 31-42 (1989).

Lim, D.Y., Yoon, J.K., and Moon, B., Interrelationship between dopaminergic receptors and catecholamine secretion from the rat adrenal gland. *Korean J. Pharmacol.,* 30(1), 87-100 (1994).

Luchsinger, A., Grilli, M., Forte, P., Morales, E., and Velasco, M., Metoclopramide blocks bromocriptine-induced antihypertensive effect in hypertensive patients. *International J. Clin. Pharmacol.Ther.,* 3, 509-512 (1995).

Lyon, R.A., Titeler, M., Bigornia, L., and Schneider, A.S., D_2 dopamine receptors on bovine chromaffin cell membranes: identification and characterization by [^{3}H] N-methylspiperone binding. *J. Neurochem.,* 48, 631-635 (1987).

Malgaroli, A., Vallar, L., Elahi, F.R., Pozzan, T., Spada, A., and Meldolesi, J., Dopamine inhibits cytosolic Ca^{2+} increases in rat lactotroph cells. *J. Biol. Chem.,* 262, 13920-13927 (1987).

Memo, M., Carboni, E., Trabucchi, M., Carruba, M.O., and Spano, P.F., Dopamine inhibition of neurotensin-induced increase in Ca^{2+} influx intro rat pituitary cells. *Brain Res.,* 347, 253-257 (1985).

Merrit, J.E., and Brown, B.L., The possible involvement of both calcium and cyclic AMP in the dopaminergic inhibition of prolactin secretion. *Life Sci.,* 35, 707-711 (1984).

Montastruc, J.L., Gaillard, G., Rascol, O., Tran, M.A., and Montastruc, P., Effect of apomorphine on adrenal medullary catecholamine levels. *Fundam. Clin. Pharmacol.,* 3(6), 665-670 (1989).

Montiel, C., Artalejo, A. R., Bermejo, P.M., and Sanchez-Garcia F., A dopaminergic receptor in adrenal medulla as a possible site of action for the droperidol-evoked hypertensive response. *Anesthesiology,* 65(5), 474-479 (1986).

ODowd, B. F., Structures of dopamine receptors. *J. Neurochem.,* 60(3), 804-816 (1993).

Quick, M., Bergeron, L., Mount, H., and Philte, J., Dopamine D_2 receptor binding in adrenal medulla: characterization using [^{3}H] spiperone. *Biochem. Pharmacol.,* 36, 3707-3713 (1987).

Roquebert, J., Alaoui, K., Moran., and Benito, A., Cardiovascular effects of bromocriptine in rats: role of peripheral adrenergic and dopaminergic receptors. *J. Auton. Pharmacol.,* 10, 85-96 (1990).

Schettini, G., Cronin, M.J., and Macleod, R.M., Adenosine 3', 5'-monophosphate(cAMP) and calcium calmodulin interrelation in the control of prolactin secretion: evidence for dopamine inhibition of cAMP accumulation and prolactin release after calcium mobilization. *Endocrinology,* 112, 1801-1807 (1983).

Schramm, M., Thomas, G., Towart, R., and Franckowiak, G., Novel dihydropyridines with positive isotropic action through activation of Ca^{2+} channels. *Nature,* 303, 535-537 (1982).

Seidler, N.W., Jona, I., Vegh, N., and Martonosi, A., Cyclopiazonic acid is a specific inhibitor of the Ca^{2+}-ATPase of sarcoplasimc reticulum. *J. Biol. Chem .,* 264, 17816-17823 (1989).

Sibley, D. R., and Monsma, F. J., Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.,* 13(2), 61-69 (1992).

Sorimachi, M., Yamagami, K., and Nishimura, S., A muscarinic receptor agonist mobilizes Ca^{2+} from caffeine and inositol-1,4,5-trisphosphate-sensitive Ca^{2+} stores in cat adrenal chromaffin cells. *Brain Res.,* 571, 154-158 (1992).

Suzuki, M., Muraki, K., Imaizumi, Y., and Watanabe, M., Cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-pump, reduces Ca^{2+}-dependent K^+ currents in guinea-pig smooth muscle cells. *Br. J. Pharmacol.,* 107, 134-140 (1992).

Tallarida, R.J., and Murray, R.B., *Manual of pharmacologic calculation with computer programs.* 2nd Ed. New York Speringer-Verlag, pp. 131-136 (1987).

Uceda, G., Artalejo, A.R., Lopez, M.G., Abad, F., Neher, E., and Garcia, A.G., Ca^{2+}-activated K^+ channels modulated muscarinic secretion in cat chromaffin cells. *J. Physiol.,* 454, 213-230 (1992).

Vallar, L., and Meldolesi, J., Mechanisms of signal transduction at the dopamine D2 receptor. *Trends Pharmacol. Sci.,* 10(2), 74-77(1989).

Van Loon, G. R., Sole, M. J., Bain, J., and Ruse, J. L., Effects of bromocriptine on plasma catecholamines in normal men. *Neuroendocrinology,* 28, 425-434 (1979).

Wada, Y., Satoh, K., and Taira, N., Cardiovascular profile of Bay-K-8644, a presumed calcium channel activator in the dog. *Naunyn-Schmiedebergs. Arch. Pharmacol.,* 328, 382-387 (1985).

Wakade, A.R., Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. *J. Physiol.,* 313, 463-480 (1981).

Ziegler, M.G., Lake, C.R., Williams, A.C., Teychenne, P.F., Shoulson, I., and Steinsland, O., Bromocriptine inhibits norepinephrine release. *Clin. Pharmacol. Ther.,* 25, 137-142 (1979).