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Interrelationship between Dopaminergic Receptors and Catecholamine Secretion from the Rat Adrenal Gland†

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It has been known for some time that dopamine-containing cells are existed in sympathetic ganglia, i.e., small, intensely fluorescent cells. However, its role and mechanism of action as a peripheral neurotransmitter are poorly understood so far. In the present study, an attempt was made to examine the effect of apomorphine, which is known to be a selective agonist of dopaminergic D_2 receptor on secretion of catecholamines (CA) from the isolated perfused rat adrenal gland.

The perfusion of a low concentration of 10 uM apomorphine into an adrenal vein for 20 min produced significant reduction in CA secretion induced by 5.32 mM ACh, 56 mM KCl, 100 uM DMPP and 100 uM McN-A-343. Increasing apomorphine concentration to 30 uM led to more markedly decreased CA secretion as compared to the case of 10 uM apomorphine and also did inhibit clearly CA release by 10^{-5} M Bay-K-8644. Furthermore, in adrenal glands preloaded with a higher dose of 100 uM apomorphine, CA releases evoked by ACh, excess K^+, DMPP and McN-A-343 were almost abolished by the drug.

The perfusion of 3.3×10^{-5} M metoclopramide, which is well-known as a selective dopaminergic D_2 antagonist, produced significantly inhibitory effect of CA release by ACh, DMPP and McN-A-343 but did not affect that by excess K^+. However, preloading of 30uM apomorphine in the presence of metoclopramide did not modify the CA secretory effect of excess K^+ and DMPP.

These experimental results demonstrate that apomorphine causes dose-dependent inhibition of CA secretion by cholinergic receptor stimulation and also by membrane depolarization from the isolated perfused rat adrenal gland, suggesting that these effects appear to be exerted by inhibiting influx of extracellular calcium into the rat adrenal medullary chromaffin cells through activation of inhibitory dopaminergic receptors.

Key Words: Dopaminergic D_2-receptors, Catecholamine secretion, Adrenal gland

INTRODUCTION

It has been known that the adrenal medulla is embriologically derived from the neural crest and shares many properties in common with sympathetic neurones, including the ability to synthetize catecholamines (CA) from L-tyrosine. Dopamine is produced during this biosynthetic process, and while it has generally been considered that dopamine is used only for the synthesis of CA, evidence obtained from a variety of peripheral nervous system tissues indicates that dopamine may have a functional role in the adrenal medulla (Bell, 1982; Goldberg and Kohi, 1983; Lackovic and Neff, 1983). Earlier work by Carlsson (1975) made hypothesis of existence of dopamine D_2 receptors on neurons and it has been suggested that these receptors were the site of action of dopamine receptor agonists which inhibit dopamine release and synthesis in the central nervous

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system. The dopamine D_2 receptor inhibition of dopamine release has been shown to occur in a number of brain areas including the striatum, olfactory tubercle and nucleus accumbens (Brase, 1980; Bowyer and Weiner, 1989; Masserano, *et al.*, 1990; Parker and Cubeddu, 1985; Starke *et al.*, 1978; Stoof *et al.*, 1980). Moreover, it has been found that dopamine D_2 receptors are localized on central and peripheral noradrenergic neurons and when activated an inhibition of norepinephrine release from these neurones (Friedman *et al.*, 1989; Fuder and Muscholl, 1978; Galzin *et al.*, 1982). The presence of dopamine receptors in adrenomedullary chromaffin cells has been widely reported. Dopamine agonists are 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 culture (Bigornia *et al.*, 1988; Bigornia *et al.*, 1990). The fact that the inhibitory effects of dopamine is reversed by specific D_2 antagonists indicates that 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).

However, more recently, Artalejo and his coworkers (1990) have identified D_1 dopaminergic receptors on bovine chromaffin cells by fluorescence microscopy and have also shown that stimulation of the D_1 receptors activates the facilitation of Ca^{++} current in the abscence of predepolarizations or repetitive activity and that activation by D_1 agonists is mediated by cyclic AMP and protein kinase A. The recruitment of facilitation of Ca^{++} channels by dopamine may from the basis of a positive feedback loop mechanism that augments CA secretion. 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 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).

Thus, it is clear that there are much contraditory and controversial reports on the modulatory effect of dopaminergic receptors in CA release from the adrenal medulla. Therefore, it seemed likely that adrenal medulla chromaffin cells could also contain a dopamine receptor capable of modulationg the Ca^{++}-dependent CA release by nicotinic cholinergic receptor stimulation. The purpose of the present study is to investigate whether a dopamine receptor agonist and an antagonist modify the release of CA evoked by cholinergic receptor- and membrane depolarization-mediated stimulation from the isolated perfused rat adrenal glands and to elucidate the mechanism of its action.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague-Dawley rats, weighing 180~300 grams, were anesthetized with ether. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order 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 cannuations.

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℃

Perfusion of adrenal gland

The adrenal glands were perfused by means of a ISCO pump (WIZ Co.) at a rate of 0.3 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; $NaHCO_3$, 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±0.05. The solution contained disodium EDTA (10 ug/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of catecholamine.

Drug administration

The perfusions of DMPP (100 uM) and McN-A-343 (100 uM) for 2 minutes and/or a single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock, and Bay-K-8644 (10^{-5} M) was also perfused for 4 min.

In the preliminary experiments, it was found that upon administration of the above drugs, secretory responses to ACh, KCl, McN-A-343 and Bay-K-8644 returned to preinjection level in about 4 min, but the responses to DMPP in 8 min. Generally, the adrenal glands perfusats was collected in chilled tubes.

Collection of perfusate

As a rule, prior to each stimulation with cholinergic agonists or excess K^+ perfusate samples were collected (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. Each perfusate was collected for 4 to 8 min. The amounts secreted in the "background sample" have been subtracted from those secrected from the "stimulated sample" to obtain the net secretion value of CA, which is showh in all of the figures.

To study the effects of dopaminergic D_2- agonist and antagonist on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing apomorphine or metoclopramide for 20 min, then the perfusate was collected for a specific time period (background sample), and then the medium was changed to the one containing the stimulating agent and the perfusates were collected for the same period as that for the "background sample".

Measurement of catecholamines

CA content of perfusate was measured directly by the flurometric method of Anton and Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using fluorospectrophotometer (Shima dzu Co.).

A volume of 0.2ml of the perfusate was used for the reaction. The CA content in the perfusate of simulated gnands by secretogogues used in the present work was high enough to obtain readings several fold 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.

All data are presented as means with their standard errors, and the significance of differences was analyzed by Student's paired t-test using the computer system as previously described (Tallarida and Murray, 1987).

Drugs and their sources

The following drugs were used: apomorphine hydrochloride, 3, 4, 5-trimethoxy benzoic acid-8-(diethylamino) octyl ester hydrochloride (TMB-8), acetylcholine chloride, 1,1-dimethyl-4-phenyl piperazimium iodide (DMPP), norepinephrine bitartrate, methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay-K-8644), metoclopramide hydrochloride (Sigma Chemical Co., U.S.A.), (3-(m-chlorophenylcarbamoyloxy)-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 except Bay-K-8644. Bay-K-8644 was dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1

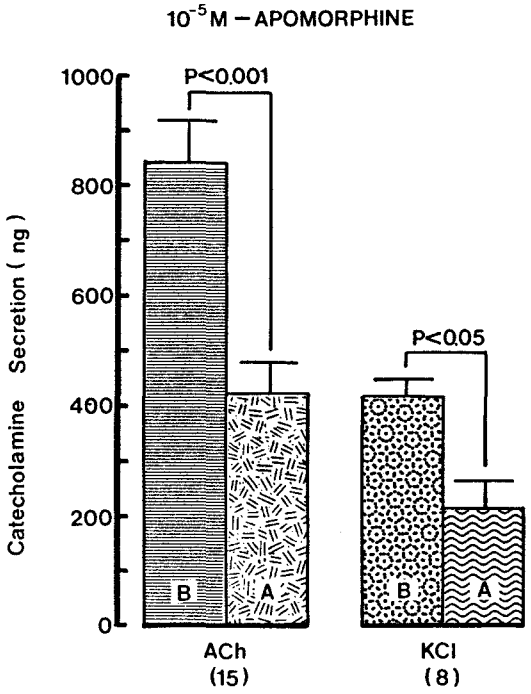

Fig. 1. Influence of 10 uM apomorphine on ACh-and excess K^+-stimulated catecholamine (CA) secretion from the isolated perfused rat adrenal glands. CA secretion was induced by a single injection of ACh (5.32 mM) and prior to initiation of the experimental protocol. "B"and "A"denote CA secretion evoked by ACh and excess KCl, before (B) and after (A) preloading with 10 μM apomorphine for 20 min, respectively. Numerals in the parenthesis indicate 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 the adrenal gland in ng. Abscissa: secretogogues. Statistical difference was obtained by comparing the control with the pretreated group. Each perfusate was collected for 4 minutes. ACh: acetylcholine

%). Concentrations of all drugs used are expressed in terms of molar base.

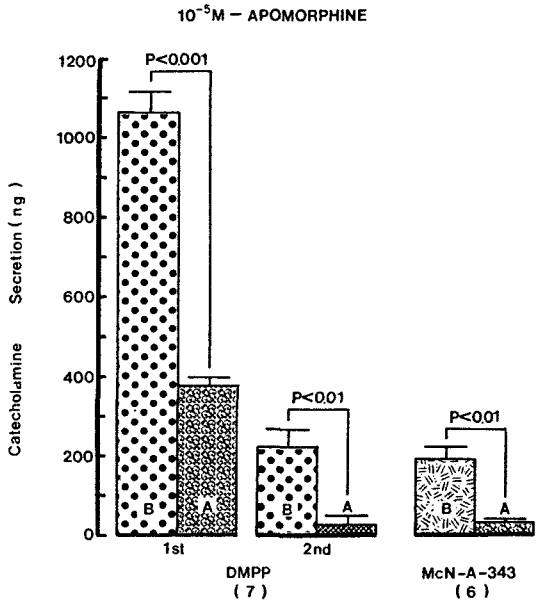

Fig. 2. Influence of 10 μM apomorphine on nicotinic and muscarinic stimulated CA secretory responses. DMPP (100 μM) and McN-A-343 (100 μM) were perfused into an adrenal vein for 2min before and after preloading with 10 μM-apomorphine for 20 min, respectively. DMPP-induced perfusates was collected twice successively for each 4 minutes but McN-A-343-induced perfusate only for 4minutes. Other legends and methods are the same as in fig. 1.

RESULTS

Effect of 10uM apomorphine of CA secretion evoked by ACh, excess K^+, DMPP and McN-A-343 from perfused rat adrenal glands

After the initial perfusion with oxygenated Krebs-bicarbonate solution for one hour, spontaneous CA release from the isolated perfused rat adrenal glands amounted to 22.5 ± 3.3 ng/2 min (n =8). Being a typical and widely used agonost for dopaminergic receptors, it was dicided initially to examine the effects of apomorphine on cholinergic receptor- as well as membrane depolarization-mediated CA secretion from perfused rat adrenal glands. Secretogogues were given at 2 hour inter-

vals. Apomorphine was present 20min before each stimulation. In the present study, it was found that apomorphine itself lacked any effect on basal CA output (Data not shown). When ACh (5.32 mM) in a volume of 0.05 ml was injected into the perfusion stream via three way stopcock, the amounts of CA secreted was 842.7±75.1 ng for 4min. However, after the preperfusion with the dopamine receptor agonist apomorphine (10 uM) for 20 min, ACh-stimulated CA secretion was significantly decreased to 428.0±52.0 ($P<0.01$, n=15) ng for 4 min as shownd in Fig. 1.

It has been found that depolarizing agent like KCl stimulates sharply CA secretion. In the present work, excess K^+ (56 mM)-stimulated CA secretion after the pretreatment with 10 uM apmorphine for 20 min was clearly reduced to 215.0±4.85 ($P<0.05$) ng/4 min as compared with its corresponding control secretion of 420.0±29.8 ng for 4min from 8 adrenal glands (Fig. 1). When perfused through the rat adrenal gland, DMPP (100 uM for 2 min), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion. As shown in Fig. 2, DMPP-stimulated CA secretion before preloading with 10 uM apomorphine was 1065.7±112.8(0~4 min) ng and 225.0±43.2 (4~8 min) ng, while after pretreatment with 10 uM apomorphine for 20 min they were greatly reduced to 380.0±20.4 (0~3 min, $P<0.001$) ng and 30.0±19.6 (4~8 min, $P<0.01$) ng, respectively from 7 rat adrenal glands.

As illustrated in Fig. 2, McN-A-343 (100 uM), which is a selective muscarinic M_1-agonist (Hammer and Giachetti, 1982), perfused into an adrenal gland for 2min caused an increased CA secretion to 195.0±29.6 ng for 4 min from 16 rat adrenal glands. However, McN-A-343-stimulated CA secretion in the presence of 10 uM apomorphine was markedly inhibited to 36.7±6.1 ($P<0.01$, n=16) ng for 4 min, which is 19% of the corresponding control secretion.

Effect of 30 uM-apomorphine on CA secretion evoked by ACh, excess K^+, DMPP, McN-A-343 and Bay-K-8644 from the perfused rat adrenal glands

In order to test the dose-dependent effects of apomorphine on receptor-mediated CA secretion as well as membrane depolarization-mediated secretion, more increased concentration of apomorphine to 30 uM was preloaded into the adrenal medulla. Figure 3 shows that 30 uM apomorphine-pretreatment diminishes greatly inhibition of CA secretion evoked by ACh and excess KCl. In the present study, ACh (5.32 mM)- and excess K^+ (56 mM)-stimulated CA secretion prior to preloading with 30 uM apomorphine were 716.8±79.9 ng and 355.0±48.0 ng for 4min, respectively. However, under apomorphine (30 uM) effect, which was perfused 20 min before stimulation was induced, they were markedly inhibited to 236.5±33.3 ($P<0.001$, n=17) ng and 163.3±28.9 ($P<0.01$, n=6) ng, respectively, which were 33% and 46% of their corresponding control secretion.

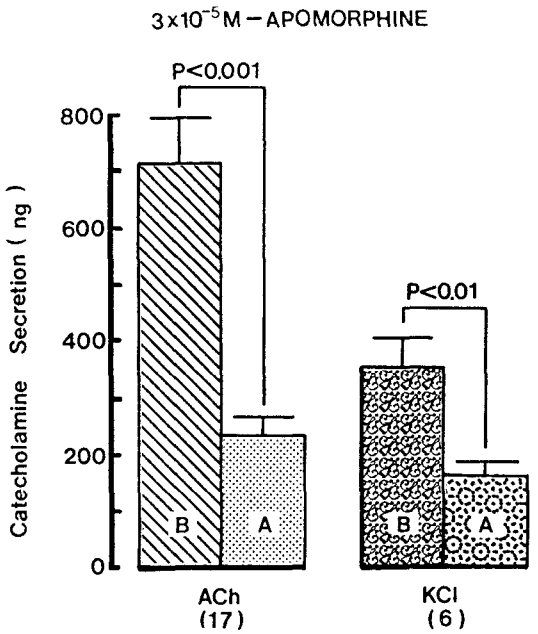

Fig. 3. Influence of 30 μM apomorphine on ACh-and excess K^+-stimulated CA secretory responses from the rat adrenal glands. Other legends and method are as in Fig. 1. and 2.

Nicotinic receptor agonist DMPP (100 uM) perfused into the adrenal gland evoked great CA secretion of 766.7±205.6 (0~4 min) ng and 141.7±46.5 (4~8 min) ng while following perfusion with 30 uM apomorphine for 20 min they were clearly reduced to 200.0±38.9 (0~4 min, $P<0.05$, n=6) ng

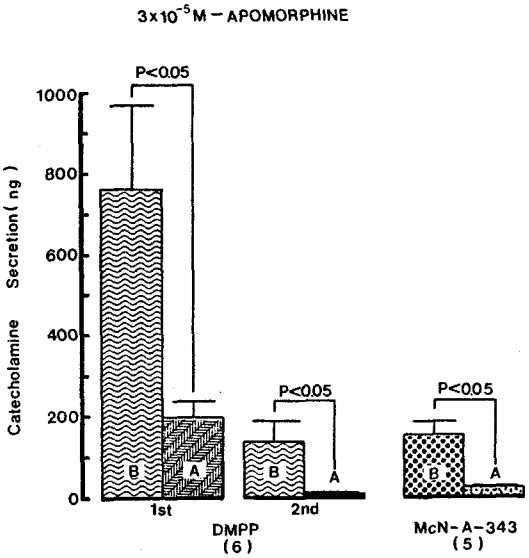

Fig. 4. Influence of 30 μM apomorphine on nicotinic and muscarinic stimulated CA secretory responses. Other legends and methods are as in Fig. 1 and 2.

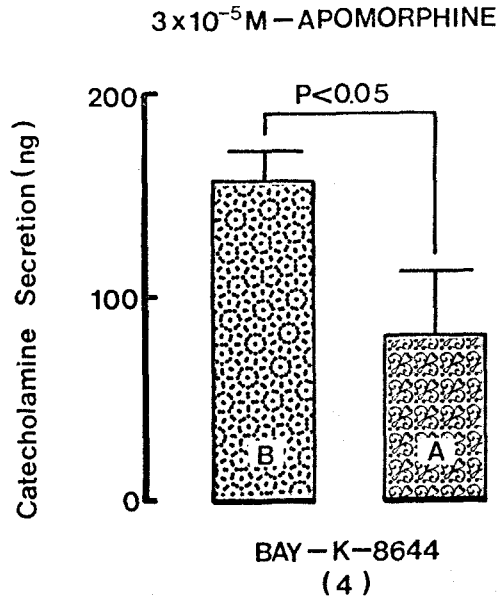

Fig. 5. Influence of 30 μM apomorphine on CA secretion evoked by Bay-K-8644. Bay-K-8644 (10 μM) was perfused into an adrenal vein for 4min before and after the pretreatment with 30 μM apomorphine for 20 min, respectively. Its perfusate was collected for 4min. Other legends and methods are the same as in Fig. 1 and 2.

and 10.0±3.8 (4~8 min, $P<0.05$, n=6) ng as compared with their control responses, respectively as shown as in Fig. 4. In 5 rat adrenal glands, McN-A-343 (100 uM)- stimulated CA secretion was 153.0±33.7 ng before administration of apomorphine but in the presence of 30 uM apomorphine McN-A-343-evoked CA-secretion was significantly decreased to 26.0±2.5 ($P<0.05$) ng of its control secretion as shown in Fig. 4. Since Bay-K-8644 is known to be a calcium channel activator and to cause positive inotropy and vasconstriction in isolated tissues and intact animals (Schramm *et al.*, 1982; Wada *et al.*, 1985) and to enhance basal Ca^{++} uptake (Garcin *et al.*, 1984) and CA release (Lim *et al.*, 1992), it was of interest to determine the effects of apomorphine on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Figure 5 illustrates the inhibitory effect of 30 uM apomorphine on Bay-K-8644-evoked CA secretion. Bay-K-8644 (10 uM) given into the perfusion stream for 4 min increased CA secretion to 157.5±13.0 ng from 4 rat adrenal glands. However, under the effect of 30 uM apomorphine, which was preloaded 20 min before Bay-K-8644 was introduced, Bay-K-8644-stimulated CA secretion was strikingly depressed to 82.5±30.0 ($P<0.05$) ng for 4min as compared to the corresponding control release; thus, the release was reduced to 52% of the control secretion.

Effect of 100 uM apomorphine on CA secretion evoked by ACh, excess K^+, DMPP and McN-A-343 from the perfused rat adrenal glands

It was to examine the effects of 100 uM apomorphine as a maximal concentration in the present experiment on cholinergic receptor- as well as membrane depolarization-mediated CA secretion from the isolated perfused rat adrenal glands. Prior to preloading with 100uM apomorphine CA secretion evoked by a single injection of ACh (5.32 mM) and excess K^+ (56 mM) in a volume of 0.05 ml into an adrenal gland was 643.4±61.6 ng and 244.7±47.2 ng for 4 min, respectively as shown as in Fig. 6. However, following the

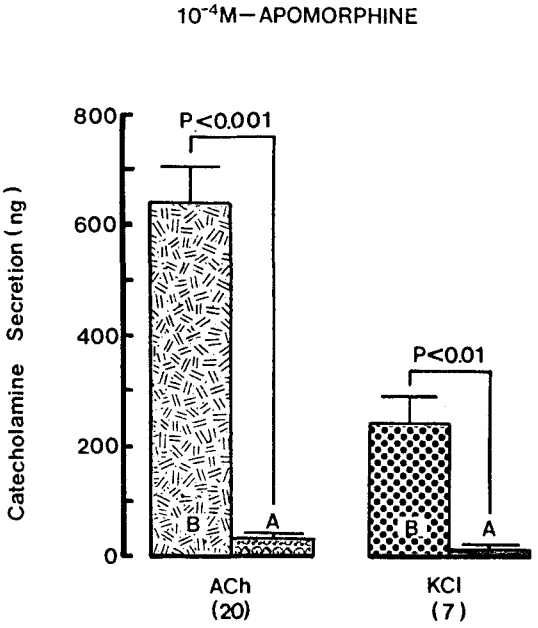

Fig. 6. Influence of 100 μM apomorphine on ACh-and excess K^+-stimulated CA secretion from the isolated rat adrenal glands. Other legends and methods are the same as in Fig. 1.

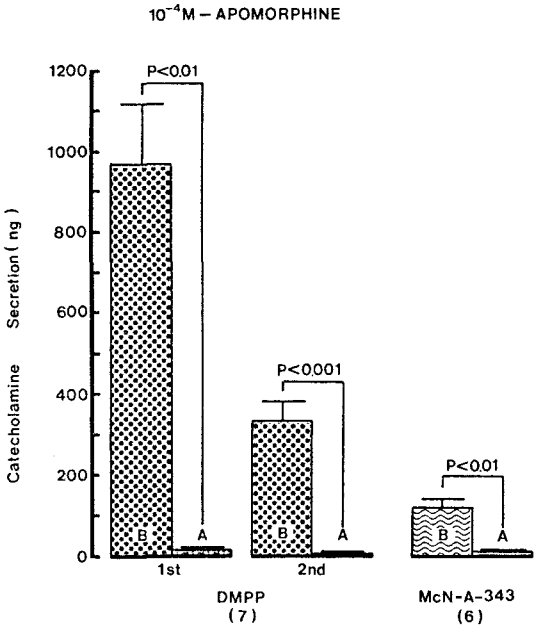

Fig. 7. Influence of 100 uM apomorphine on nicotinic and muscarinic stimulated CA secretory responses. Other legends and methods are the same as in Fig. 1.

preloading with 100 uM apomorphine for 20 min they were almost completely blocked to 32.3±8.5 ($P<0.001$, n=20) ng and 14.3±8.4 ($P<0.01$, n=7) ng which were 5% and 5.8% of their corresponding control secretion, respectively (Fig. 6). Fig. 7 shows the almost blockade of 100 uM apomorphine to CA secretory effect evoked by DMPP and McN-A-343 from the rat adrenal glands. In the present work, in the absence of apomorphine, DMPP (100 uM)- and McN-343 (100 uM)-evoked CA secretion amounted to 971.4±194.8 (0~4 min) ng and 337.2±42.2 (4~8 min) ng, and 120.0±23.8 (0~4 min) ng, respectively while in the presence of 100 uM apomorphine which was preloaded 20 min before stimulation they were prominently depressed to 17.1±3.8 (0~4 min, $P<0.01$, n=7) ng and 8.6±2.0 (4~8 min, $P<0.001$, n=7) ng and 10.0 ±3.1 (0~4 min, $P<0.01$, n=6) ng, respectively.

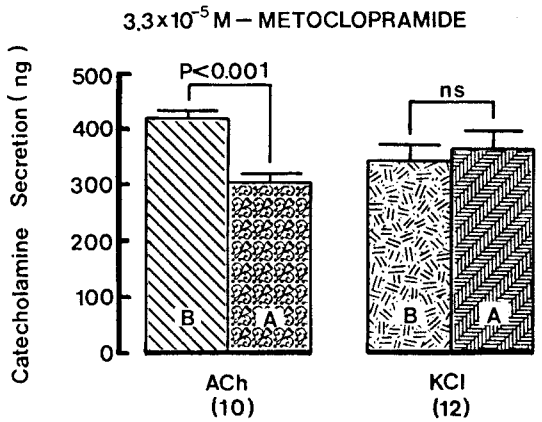

Fig. 8. Influence of metoclopramide on ACh- and excess K^+-stimulated CA secretion from the rat adrenal glands. ACh (5.32 mM) and excess KCl (56 mM) were given into an adrenal vein before and after the perfusion with metoclopramide (3.3×10^{-5} M) for 30 min, respectively. Other legends and methods are as in Fig. 1 and 2. ns: statistically nonsignificance.

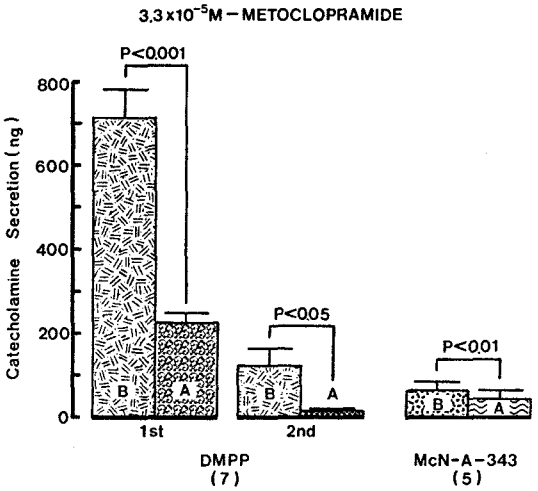

Fig. 9. Influence of metoclopramide on nicotinic and muscarinic stimulated CA secretory responses. Other legends and methods are as in Fig. 1 and 2.

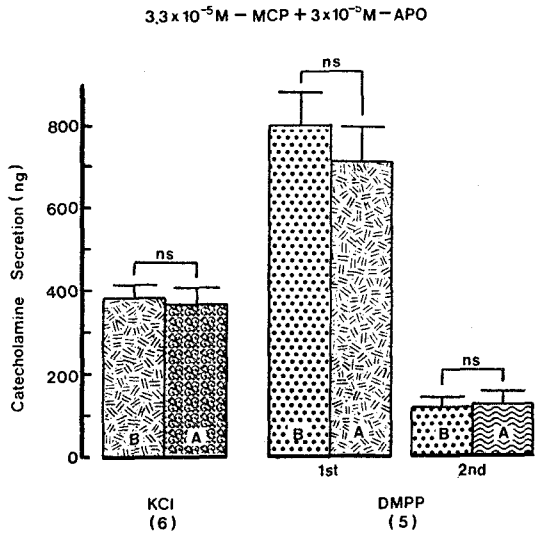

Fig. 10. Influence of 30 μM apomorphine in the presence of metoclopramide (33 μM) on excess K^+- and DMPP-stimulated CA secretory responses. Excess KCl (56 mM) and DMPP (100 μM) were perfused into an adrenal vein before and after preloading with 30 μM apomorphine plus 33 μM metoclopramide for 20 min, respectively. Other legends and methods are the same as in Fig. 1 and 2. APO: apomorphine, MCP: metoclopramide

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

As shown in the previous experimental results, it was found that apomorphine produced the dose-dependent inhibition of cholinergic receptor- and membrane depolarization-mediated CA secretion. Therefore, it was of interest to examine the effect of metoclopramide, which is known to be a selective dopamine D_2-receptor antagonist, on CA secretory action evoked by ACh, excess K^+, DMPP and McN-A-343 from the isolated perfused rat adrenal glands. CA secretion stimulated by ACh (5.3 mM) and excess K^+ (56 mM) after preloading with 3.3×10^{-5} M metoclopramide for 30 min amounted to 305.6 ± 13.0 ($P<0.001$, $n=10$) ng and 367.5 ± 27.0 (ns, $n=12$) ng, respectively as compared to each corresponding control secretion of 420 ± 13.8 ng and 341.3 ± 28.0 ng as shown in Fig. 8. DMPP (100 uM)- and McN-A-343 (100 uM)-stimulated CA secretions in the absence of metoclopramide were 715.7 ± 69.1 (0~4 min) ng and 126.4 ± 36.3 (4~8 min) ng and 66.0 ± 21.5 (0~4 min) ng, respectively. However, after preloading with 3.3×10^{-5} M metoclopramide for 30 min they were significantly diminished to 227.1 ± 21.6 (0~4 min, $P<0.001$, $n=7$) ng and 10.7 ± 5.3 (4~8 min, $P<0.05$, $n=7$) ng and 45.0 ± 22.7 (0~4 min, $P<0.01$, $n=3$) ng as compared with their corresponding control release. Fig. 9 illustrates that metoclopramide inhibits markedly CA secretion stimulated by DMPP and McN-A-343.

Effect of metoclopramide on the inhibition by apomorphine of CA secretory responses stimulated by excess K^+ and DMPP

Since it has been reported that 10^{-6}M dopamine-induced inhibition of release of [^{3}H] norepinephrine from isolated perfused rabbit adrenal glands could be reversed completely by the dopamine D_2 selective antagonist 3×10^{-6} M metoclopramide (Collet and Story, 1982; Artalejo *et al.*, 1985), it was interesting to define whether metoclopramide is able to reverse the inhibitory

effect of apomorphine on nicotinic and membrane depolarization-evoked CA secretory responses from perfused rat adrenal glands.

When perfused into an adrenal vein in a volume of 0.05 ml, excess K^+ (56 mM) caused CA secretion of 385.2±29.5 ng for 4 min but in the presence of 30 uM apomorphine along with 3.3×10^{-5} M metoclopramide excess K^+-induced CA secretion amounted to 370.1±42.6 (ns) ng for 4min from the 6 rat adrenal glands as shown in Fig. 10.

DMPP (100 uM)-stimulated CA secretion in the presence of 30 uM apomorphine plus 3.3×10^{-5} M metoclopramide was 711.5±83.6 (0~4 min, ns) ng and 126.2±30.5 (4~8 min, ns) ng as compared to their control secretions of 805.4±76.2 (0~4 min) ng and 118.3±24.8 (4~8 min) ng from 5 rat adrenal glands, respectively (Fig. 10).

DISCUSSION

The present experimental results obtained so far indicate that the dopaminergic receptor agonist apomorphine strongly inhibits CA secretion stimulated by ACh, excess K^+, DMPP, McN-A-343 and Bay-K-8644 from the isolated perfused rat adrenal glands, and that apomorphine itself does not affect basal CA release. In the presence of the dopamine receptor antagonist metoclopramide, apomorphine did not inhibit CA releases evoked by DMPP- as well as excess K^+-stimulation. However, metoclopramide alone considerablely depresses CA secretory effects evoked by nicotinic and muscarinic stimulation without modification of CA releasing effect caused by excess K^+-evoked deoplarization. It could be concluded that apomorphine causes the dose-dependent inhibition of CA secretory responses by cholinergic receptor stimulation and also by membrane depolarization, and that these effects of apomorphine seem to be due to inhibition of extracellular calcium influx into the rat adrenal medullary chromaffin cells through activation of inhibitory dopaminergic receptors. In support of these experimental results that apomorphine in the present work inhibits CA secretion evoked by nicotinic, muscarinic and depolarization-mediated stimulation through activation of inhibitory dopa-minergic D_2 receptor in adrenal medullary chromaffin cells of the rat, it has been found that the presence of D_2 dopamine receptors on adrenal chromaffin cells in demonstrated in recent studies by radioligand binding methods (Gonzalez *et al.*, 1986; Lyon *et al.*, 1987; Quick *et al.*, 1987). These dopamine receptors on chromaffin cells appears to function as an inhibitory modulator of adrenal CA secretion as shown by the results obtained in the cultured bovine adrenal chromaffin cells (Bigornia *et al.*, 1988: 1990) and by recent studies with perfused cat adrenal glands (Artalejo *et al.*, 1985; Gonzalez *et al.*, 1986; Montastruc *et al.*, 1989). Artalejo and his coworkers (1985) have shown that cat adrenal medulla chromaffin cell membrane contains a dopaminergic receptor which modulates the CA secretory process triggered by stimulation of the nicotinic cholinoceptor, and that dopamine is released in measurable amounts, together with adrenaline and that dopamine is released in measurable amounts together with adrenaline and noradrenaline from perfused cat adrenal glands in response to nicotinic stimulation, favouring a role for this dopaminergic in modulating CA release from the chromaffin cells. Moreover, it has been found that as in the cat, the bovine adrenal glands contain dopaminergic receptors that modulates CA secretion evoked by stimulation of the nicotinic cholinergic receptors through activation of the inhibitory D_2 type receptors (Gonzalez *et al.*, 1986). Recently, Montastruc *et al.* (1989) have reported that subcutaneous injection of apomorphine in normotensive rats produces a dose-dependent decrease in CA content of the adrenal gland via the activation of D_2 dopamine receptor probably located on splanchnic nerve endings. The investigational data obtained in the bovine adrenal chromaffin cells could support enoughly that D_2 dopamine 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 and Story, 1982a: 1982b; Artalejo *et al.*, 1985; Gonzalez *et al.*, 1988; 1990; Montiel *et al.*, 1986; Montastruc *et al.*, 1989). In the present ex-

periment, the findings that apomorphine in the presence of metoclopramide did not modify the CA secretory process evoked by excess K^+ and DMPP conform that apomorphine inhibits CA secretory responses stimulated by nicotinic as well as depolarization-mediated stimulation through activation of inhibitory dopaminergic D_2-receptors on adrenal medullary chromaffin cells of the rat. These previous results are consistent with those of the present study. However, in the present work, the fact that apomorphine produces a dose-denpendent inhibition of CA secretion by McN-A-343, which is known to be a selective muscarinic M_1-agonist, suggests new other concept that apomorphine can modulate the CA secretory process induced by activation of muscarinic M_1-receptors as well as nicotinic receptors in the rat adrenal medulla. Collet and Story (1982a) found that 10^{-6} M dopamine inhibited the electrically evoked release of [^{3}H] norepinephrine from isolated perfused rabbit adrenal glands. This inhibition could be reversed completely by the dopamine D_2 selective antagonist metoclopramide (3×10^{-6} M). Curiously, perfusion with 33 uM metoclopramide only for 30min in the present study depressed CA secretion evoked by ACh, DMPP and McN-A-343 but did not that by excess K^+. In previous experiments, it has been known that metoclopramide causes CA secretion in the perfused rat adrenal gland (Lim *et al.*, 1989). It seems that the contradictory effect of metoclopramide on CA secretory effect may be due to the range of concentration or to the other unknown mechanism of action of CA secretory precess. However, in support of this idea, it has been found that the highest concentration (50 uM) of droperidol, a dopaminergic D_2 antagonist abolishes the adrenal catecholaminergic secretion evoked by nicotine in the isolated cat adrenal glands perfused with Krebs-bicarbonate solution. The mechanism of this effect is probably related to an interference of droperidol with calmodulin, an intracellular calcium binding protein that plays an important role in regulating many physiologic processes, including the secretory event (Montiel *et al.*, 1986). In fact, it has been shown that several butyrophenones, like haloperidol, have an inhibitory effect of calmodulin-dependent processes such as phosphodiesterase activity with a median inhibitory concentration (IC_{50}) of 60 uM (Weiss *et al.*, 1982). Instead, when lowest concentration (0.05 uM) of droperidol was used, both a reversal of the inhibitory effect of apomorphine and a facilitatory effect on the CA secretory response induced by nicotine through the removal of inhibitory dopaminergic mechanism present in the chromaffin cells were observed (Montiel *et al.*, 1986). This concentration is similar to those used by Steinsland and Hiedle (1978) to explore the dopaminergic antagonist action of haloperidol in the rabbit ear central arteries and is in ranges of the calculated droperidol plasma concentrations. Such a mechanism could occur in an exaggerated manner in patients with pheochromocytoma.

Thus, the present experimental data indicate that apomorphine causes the inhibitory effect of CA secretory process evoked by nicotinic and muscarinic receptors-mediated stimulation through D_2 dopaminergic activation because apo-morphine in the presence of metoclopramide did not affect CA secretory effect of DMPP or excess KCl, although the effect of D_1 receptors was not examined in this study. This inhibitory D_2 dopaminergic effect has been shown not to interact D_1 receptors as described previously (Bavasta *et al.*, 1986; Bigornia *et al.*, 1988b; 1990). Consistent with the present results, dopaminergic inhibitory effects in other systems were found to be mediated specifically by the D_2-receptor subtype (Memo *et al.*, 1985; de Vlieger *et al.*, 1985; Cooper *et al.*, 1986; Malgaroli *et al.*, 1987). Moreover, Bigornia et al. (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. The present work also illustrates that apomorphine produces the inhibitory modulation of adrenal CA secretion at by stimulation of the inhibitory dopminergic D_2 receptors at least partly through inhibition of calcium channel currents. The findings that apmorphine significantly inhibited CA secretory responses evoked by excess K^+ depolarization as well as by Bay-K-8644, which specifically activates an L-type, voltage-sensitive calcium channel, in the present investigation demonstrate that the inhibitory effect of apomorphine on CA release is due to the blockade of the volt-

age-sensitive calcium channel. In support of these idea, there are now sizeable lituratures demonstrating a key role of calcium influx through voltage-sensitive Ca^{++} channels as a physiological pathway for activation of adrenal CA secretion (Douglas, 1975; Aguirre *et al.*, 1977; Schneider *et al.*, 1977; 1981; Holz *et al.*, 1982; Kilpatrick *et al.*, 1982; Cena *et al.*, 1983; Knight and Kestevan, 1983; Kao and Schneider, 1986). Bigornia and his coworkers (1988) showed that apomorphine caused a dose-dependent inhibition of $^{45}Ca^{++}$ uptake stimulated by excess K^+ depolarization and that by Bay-K-8644, a calcium channel activator. These effects are almost completely in agreement with the present experimental data. In view of the results so far obtained from this 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 receptor-mediated inhibition of CA secretion. This would be consistent with findings in several other systems. However, in contrast with the present experimental results, Huettl and his colleagues (1991) have concluded 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 agonist pergolide as well as apomorphine on CA release from the chromaffin cells was not reversed or antagonized by the selective dopaminergic D_2 receptor antagonists haloperidol, domperidone, metoclopramide, fluphenazine, flupentixol, (+)- or (−)-sulpiride, the dopaminergic D_1 receptor antagonist SCH 23390 or the alpha receptor antagonist phentolamine. It is thought that apomorphine and pergolide act in a nonreceptor-mediated menner to inhibit CA release from bovine chromaffin cells. In addition, it has been shown that stimulation of the D_1 receptors on bovine chromaffin cells activates the facilitation of Ca^{++} currents in the abscence of predepolarizations or repetitive activity, and that activation by D_1 agonists is mediated by cyclic AMP and protein kinase A. The recruitment of facilitation of Ca^{++} channels by dopamine may form the basis of a positive feedback loop mechanism that augment CA secretion (Artalejo *et al.* 1990). Under in vivo conditions (in anesthetized or conscious normal intravenously adminstered dogs), it was found that quinpirole (or apomorphine), which is known to be a selective D_2 dopaminergic agonist, showed a peripheral depressor action and a central pressor component involving an increase in both sympathetic tone and vasopressin release as described by Damaes-Michel and his coworkers (1990). They also demonstrate clearly that peripheral dopaminergic D_2 receptors are not involved in the control of CA release from the adrenal medulla.

Thus, although there are considerable reports contrasted with the present results, the present experimental results illustrate that apomorphine causes the inhibitory modulation of CA secretion evoked by cholinergic receptor stimulation and/or membrane depolarization through the inhibiton of calcium influx into the chromaffin cells by stimulation of the inhibitory dopamin-ergic receptors from the isolated perfused rat adrenal glands.

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=국문초록=

흰쥐 부신에서 카테콜아민 분비작용과 도파민 수용체간의 상관성

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도파민 함유세포가 교감신경절에 존재하는 것으로 알려져 있으나, 말초에서 신경전달 물질로써 그의 역할과 작용기전에 대해서 아직까지 알려진 바가 많지 않다. 따라서 본 연구에서는 도파민 D_2-수용체의 선택적인 효능약으로 알려진 apomorphine이 흰쥐 적출 관류 부신에서 카테콜아민 (CA) 분비작용에 미치는 영향을 연구코자 시도하여 다음과 같은 연구결과를 얻었다. 10 μM Apomorphine의 비교적 낮은 농도를 부신정맥내에 20분간 관류 하였을때 5.32 mM ACh, 56 mM KCl, 100 μM DMPP 및 100 μM McN-A-343 등의 투여에 의한 CA 분비작용이 의의 있게 감소되었다. Apomorphine 농도를 30 μM로 증가시켜 관류하였을때 상기약물에 의한 CA 분비작용은 더욱 억제되었으며 또한 Bay-K-8644에 의한 100 μM의 고농도로 전처치 하였을때, ACh, excess K^+, DMPP 및 McN-A-343에 의한 CA 분비작용은 현저히 차단되었다.

도파민 D_2-수용체 차단제인 metoclopramide (33 μM)으로 20분간 관류 하였을때 ACh, DMPP 및 McN-A-343에 의한 CA 분비작용은 유의하게 억제된 효과를 나타내었으나 excess K^+에 의한 CA 분비작용은 별다른 영향을 받지 않았다. 그러나 metoclopramide (30 μM) 존재하에서 30 μM apomorphine으로 20분간 전처치 하였을때 excess K^+ 뿐만 아니라 DMPP의 CA 분비작용은 별다른 변화를 받지 않았다.

이상과 같은 실험 연구결과를 종합하여 보면, apomorphine은 cholinergic receptor stimulation과 membrane depolarization에 의한 CA 분비작용을 용량의존적으로 억제하여, 이러한 작용은 억제성 도파민 수용체를 활성화 시킴으로써 흰쥐 부신 수질의 chromaffin cell 내로 칼슘의 유입을 억제하여 나타나는 것으로 사료된다.