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Studies on Secretion of Catecholamines Evoked by Metoclopramide of the Rat Adrenal Gland*

Dong Yoon Lim**, Kyu-Hyeong Kim, Cheol Hee Choi, Ho-Jin Yoo,
Dong Joon Choi and Eun-Hwa Lee
Department of Pharmacology, College of Medicine, Chosun University, Kwang Joo 501-759, Korea

ABSTRACT

The effect of metoclopramide (MCP), which is well-known as a selective dopaminergic antagonist used in treating esophageal refulx, gastroparesis and emesis induced by anticancer chemotherapy, on secretion of catecholamines (CA) in the perfused isolated rat adrenal gland was investigated.

MCP given into an adrenal vein produced the dose-related increase in CA secretion from the adrenal gland. The secretory effect of CA evoked by MCP was inhibited markedly by atropine-pretreatment. but only partially blocked when chlorisondamine was added. The secretion of CA induced by MCP was potentiated by pretreatment with physostigmine, adenosine or ouabain. However, MCP-induced CA secretion was suppressed significantly by perfusion of calcium-free Krebs solution containing 5 mM-EGTA for 30 min. Perfusion of MCP (200 ug/30 min.) attenuated the secretory effect of CA evoked by potassium chloride or acetylcholine.

These experimental results demonstrate that metoclopramide releases CA significantly by a calcium-dependent exocy totic mechanism. It is thought that the secretory effect of metoclopramide is due to activation of cholinergic muscarinic receptors present in the adrenal gland rather than nicotinic receptors and partly to the direct action on the chromaffin cell itself.

Key Words: Metoclopramide, Adrenal Gland, Catecholamines-Secretion

INTRODUCTION

It has been described that metoclopramide (MCP) is a dopaminergic antagonist that enhances motility of the upper gastrointestinal tract and depresses the vomiting center (Besancon *et al.*, 1964; Pinder *et al.*, 1976). The effect of MCP (methoxy-2-chloro-5-procainamide) makes it useful as a routine antiemetic preventing emesis induced by antineoplastic drugs, particularly cisplatin. And MCP's gastrointestinal smooth muscle stimulatory effects are related to its ability to antagonize the inhibitory neurotransmitter, dopamine; to augment acetylcholine (Ach) release and sensitize the muscarinic receptors of the gastrointestinal smooth muscle; and to coordinate gastric-pyloric-small intestinal motor function (Albibi and McCallum, 1983).

The actions of MCP are so unique and so diverse that they have generated hopes for diagnostic and therapeutic applications of this drug in many fields of medicine (Pinder *et al.*, 1976).

It has been tried against hiccup, vertigo, and orthostatic hypertension (Pinder *et al.*, 1976; Kuchel *et al.*, 1980). Kuchel *et al.*, (1980) reported that MCP improved the postural hypotension. This effect was known to be due to inhibitory effect on the vasodilatory and natriuretic action of the excessively released dopamine (Goldberg, 1974); the additional effect may have been due to shifting the adrenal medullary compensatory response from dopamine to epinephrine (Kuchel, 1980).

It has been also shown that dopaminergic stimulation by bromocriptine produces hypotensive action (Stumpe *et al.*, 1977) while dopaminergic inhibition by sulpiride induces the hypertensive action (Corvol *et al.*, 1974). It was found that a hypertensive crisis associated with evidence of catecholamines (CA) release was induced following intravenous administration of MCP in a woman with pheochromocytoma (Plouin *et al.*,

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**To whom reprint requests should be addressed.

1976; Abe *et al.*, 1984; Agabiti-Rose *et al.*, 1977). Abe *et al.*, (1984) suggested that the mechanism of hypertensive crisis induced by MCP may be due rather to its presynaptic dopaminergic blocking effect which would indirectly release CA than to the direct CA-releasing effect.

Recently, Lim *et al.*, (1988) have found that MCP causes markedly a dose-related fall in blood pressure followed by secondary transient pressor responses, and that the hypotesive activity may be due to adrenergic alpha-receptors blockade, and that the pressor activity may be exerted through stimulation of cholinergic nicotinic receptors in autonomic ganglia.

Therefore, it seems to be very interesting to test whether MCP, a dopaminergic antagonist, can cause secretion of CA in the isolated perfused adrenal gland of the rat or not, and to elucidate the mechanism of its action.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague dawley rats, weighing 180~300 g, were anesthetized with ether. The adrenal gland was isolated by a modification of previous method of 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 portions 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 to obtain enough working space for tying blood vessels and for cannultions.

As shown in Fig. 1, a cannula, used for perfusion of the adrenal gland (A), was inserted into the distal end of the renal vein after all the branches of the adrenal vein, the renal vein (if any), vena cava and aorta were ligated. Heparine (400 IU/ml) was injected into vena cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite the entrance of the adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit of the adrenal gland. 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℃ (B).

Perfusion of the adrenal gland

The adrenal glands were perfused by means of a ISCO pump (WIZ Co.) at a rate of 0.4 ml/min. The perfusion was carried out with Krebs-

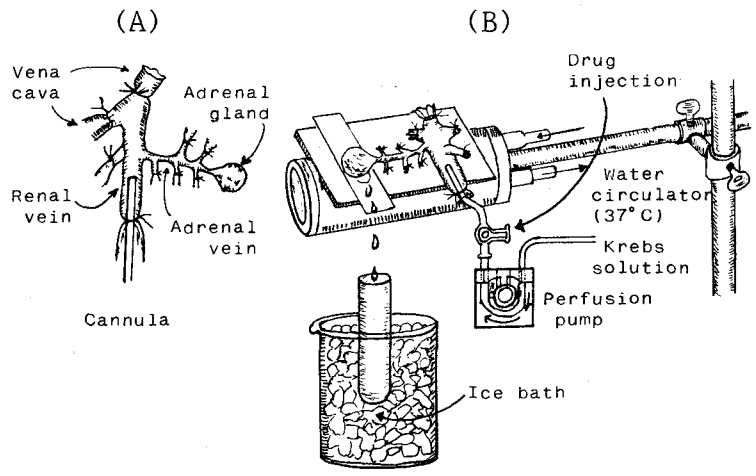

Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamine in the isolated perfused adrenal gland of the rat.

bicarbonate solution of the 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 bubbled with 95% $O_2 \pm 5\%$ CO_2, and the final pH was 7.4 ± 0.5. The solution contained disodium EDTA (10 ug/ml) and ascorbic acid (100 ug/ml.) to prevent oxidation of catecholamine.

Metoclopramide or Acetylcholine Injection

Three different concentrations(50, 100 and 200 ug) or a single concentration of Ach (50 ug) were injected in a volume of 0.05 ml into the perfusion stream via a three way stopcock (Fig. 1). In the preliminary experiments it was found that upon injection of the above doses of MCP or Ach the secretory response returned to preinjection level in about 4 min. Therefore, each sample was collected for 4 min after injection of MCP or Ach. Generally, the adrenal gland was perfused with normal Krebs solution for about one hour. The adrenal perfusate was collected in chilled tubes. Details of the collection of samples are given in Results section.

Analysis of CA

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayere (1962) without the intermediate purification on alumina, using fluorospectrophotometer (Shimazu Co.). A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by Ach or MCP was high enough to obtain readings several-fold greater than the readings 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 differences were compared by Student' s paired "t" test.

Drugs and chemicals

The following drugs and chemicals were used in this experiment: acetylcholine chloride, adenosine, norepinephrine bitartrate, physostigmine sulfate, ouabain octahydrate and EGTA (Sigma Chemical Co.), and atropine sulfate (Merk Co.) and chlorisondamine chloride (CIBA Co.). Metoclopramide HCl was obtained from Dong-A Pharmaceutical Manufacturing company (Korea). Drug concentrations are expressed as molar values except the case of MCP or Ach.

RESULTS

The release of CAs evoked by MCP

Table 1 shows that MCP or Ach caused a significant increase of the output of CA from the the isolated perfused rat adrenal gland. About one hour after perfusion of the adrenal gland with Krebs solution the gland was stimulated with 50 ug-Ach, which resulted in 384.7 ± 21.7 ug/min in secretion of CA from 49 experiments. Injection of 50 ug-MCP into the perfusion stream also produced significant secretion of CA over the back ground secretion, which was 98.3 ± 27.1 ug/4 min. A gradual increase in MCP administration resulted in greater amounts of CA to appear in the perfusate. After injection of 100 ug and 200 ug-MCP CA of secretion were 286.7 ± 31.5 ug and 366.6 ± 50.8 ug/4 min, respectively. Figure 2 shows

Table 1. Secretion of catecholamines evoked by metoclopramide from the isolated perfused rat adrenal gland

Type of stimulus to evoke secretion	Dosage of Adm. (ug)	Secretion of catecholamines (ug/4 min)	Number of animals
Acetylcholine	50	384.7 ± 21.7	49
Metoclopramide	50	98.3 ± 27.1	49
	100	286.7 ± 31.5	49
	200	366.6 ± 50.8	49

Result obtained are expressed with mean $\pm$ S.E. Thirty minutes after perfusion with Krebs solution, the adrenal gland was stimulated in each experiment with three concentrations of metoclopramide, or with single concentration of acetylcholine. The perfusate was collected for 4 min, when the adrenal gland was stimulated by acetylcholine or metoclopramide.

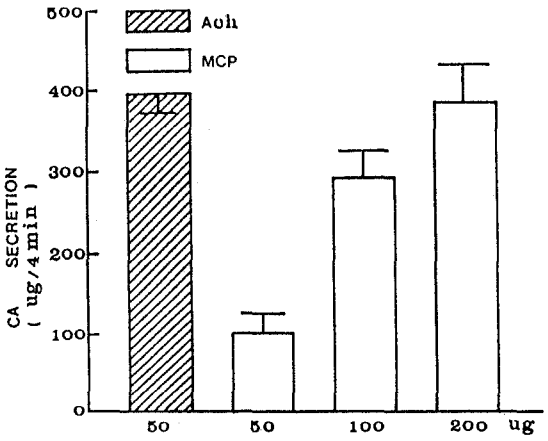

Fig. 2. Release of catecholamines from the isolated perfused rat adrenal gland following injection of metoclopramide or acetylcholine. Adrenal renal glands were perfused with Krebs solution for 30 min, and then various concentrations of metoclopraminde and a 50 ug-acetylcholine were injected at 10 min intervals into the perfusion stream. The adrenal perfusates were collected for 4 min. The averaged values were obtained from 47 to 51 experiments. Vertical lines represent S.E. of mean. Each gland was challenged with increasing order of MCP concentrations. Abscissa: doses of MCP or Ach (ug). Ordinate: secretion of catecholamines.
Ach: acetylcholine, MCP: metoclopramide, CA: catecholamines.

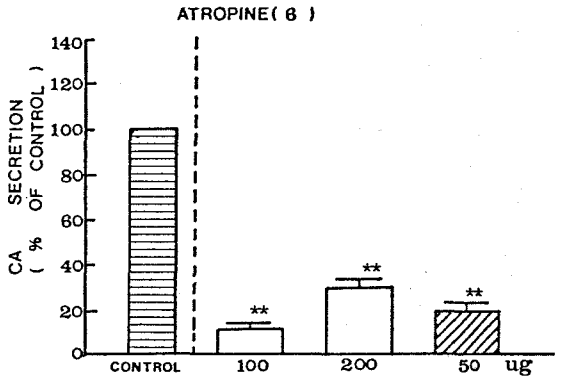

Fig. 3. The effect of atropine on secretion of catecholamines evoked by MCP or Ach. Secretion of catecholamines was produced 30 min after the beginning of perfusion with Krebs solution containing 2.3 uM-atropine. Numeral in the upper bracket shows number of animals used in this experiment. Other legends and methods are the same as in Fig. 2.
**: $p<0.01$

the relationship between the doses of MCP and secretion of CA. CA secretion was increased in proportion to the increase in MCP administration. In all subsequent experiments, 100 ug and 200 ug-MCP were used with 50 ug-Ach in order to compare each other in CA secretion.

Effect of atropine on the release of CAs induced by MCP

It is well-known that atropine reduces or abolishes its upper gastrointestinal smooth muscle action evoked by MCP (Eisner, 1968; Kowalewski and Kalode, 1975; Beani *et al.*, 1970). Therefore, MCP could release Ach from the presynaptic sites in the adrenal medulla partly through the stimulation of muscarinic receptors. It appears to be very interesting to test the influence of atropine on CA secretion evoked by MCP whether it has the muscarinic activity in the rat adrenal medulla or not.

In the present study, the secretory effect of MCP was evoked in the gland pretreated with 2.3 uM-atropine, antimuscarinic agent for 30 min. Figure 3 represents MCP or Ach-induced CA secretions were markedly inhibited by atropine-treatment. In 6 rats, responses to 100 ug and 200 ug-MCP in the presence of atropine were reduced by $84.6\pm9.61\%$ ($p<0.01$) and $71.7\pm21.67\%$ ($p<0.01$) of the corresponding control values (100%), respectively. Ach-evoked CA secretion was also decreased significantly.

Effect of chlorisondamine on the release of CAs induced by MCP

In view of the fact that MCP-induced CA secretion was inhibited clearly by treatment with atropine as shown in figure 3, the release of CA evoked by MCP could be secondary to the release of Ach produced from the presynaptic cholinergic nerve terminals present in the adrenal medulla. Figure 4 represents the effect of chlorisondamie on CA secretion evoked by MCP or Ach in order to examine whether MCP has the nicotinic activity in

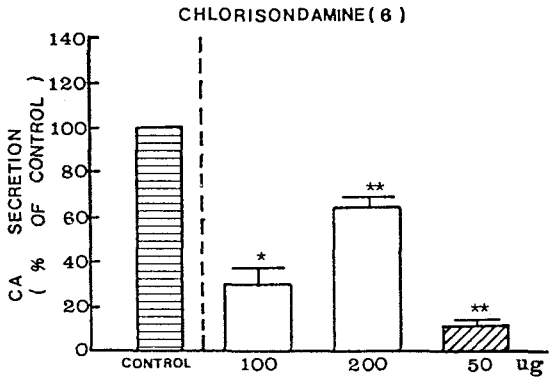

Fig. 4. The effect of chlorisondamine on secretion of catecholamines evoked by MCP or Ach. Catecholamines-secretion was evoked 30 min following the beginning of Krebs solution containing 1.0 uM-chlorisondamine. Other legends and methods are as in Fig. 2 and 3.
*: $p<0.05$, **: $p<0.01$

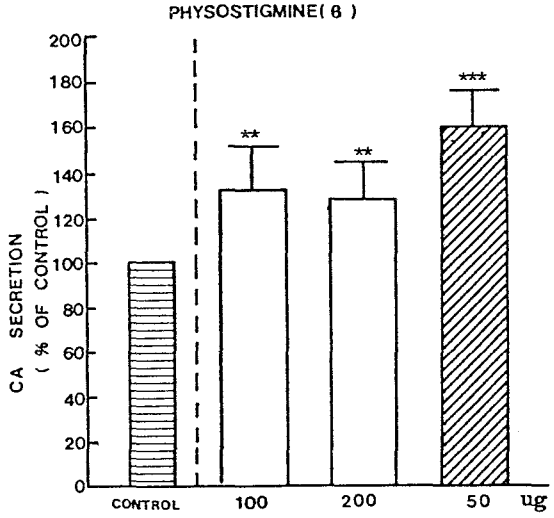

Fig. 5. The effect of physostigmine on the release of catecholamines induced by MCP or Ach. Secretion of catecholamines was caused 30 min after the beginning of perfusion with Krebs solution containing 10 nM-physostigmine. Other legends and methods are same as in Fig. 2 and 3.
**: $p<0.01$ ***: $p<0.001$

the adrenal gland or not. The gland was perfused with Krebs solution in the presence of autonomic ganglionic blocking agent, 1.0 uM-chlorisondamine (Gilman *et al.,* 1985) for 30 min. CA release by 100 ug and 200 ug-MCP, and 50 ug-Ach were markedly inhibited by 30.6 ± 13.35 ($p<0.05$) %, 35.17 ± 13.28 ($p<0.01$) % and 87.5 ± 11.20 ($p<0.01$) % of each corresponding control response, respectively.

Effect of physostigmine on the release of CAs by MCP

MCP could be releasing Ach from presynaptic sites in the adrenal medulla because MCP-evoked CA secretion was significantly inhibited by atropine-pretreatment, but the Ach released might be rapidly degraded by acetylcholinesterase. Therefore, the secretory effect of MCP was tested in glands pretreated with 10 uM-physostigmine, an anti-acetylcholinesterase (Goodman *et al.,* 1985). Figure 5 shows that MCP-evoked CA release was markedly increased in the gland treated with physostigmine than in the control gland and Ach-evoked CA release was also greatly higher. CA output induced by 100 ug and 200 ug-MCP were enhanced to $153.2\pm21.7\%$ ($p<0.01$) and $151.5\pm16.46\%$ ($p<0.01$) of each control, respectively. Ach-evoked CA release was also increased to $160.2\pm19.1\%$ ($p<0.01$) of the corresponding control.

Effect of MCP infusion on the CAs release induced by KCl or Ach

In the light of the fact that MCP-induced CA secretion was attenuated significantly by pretreatment with atropine and potentiated by physostigmine as shown in figure 3, 4 and 5, it is felt that MCP may provoke the secretory effect of CA by muscarinic activity in adrenal gland. Therefore, it seemed to be interesting to examine whether MCP will be able to modify Ach-or KCl-effects in secretion of CA or not. CA secretion evoked by Ach or KCl after perfusion of MCP (200 ug/30 min) for 30 min is shown as in figure 6 from 9 rats. CA releases induced by 180 and 300 ug-KCl, and 50 ug-Ach were inhibited markedly by 72.0 ± 8.67 % ($p<0.01$), 72.7 ± 10.27 ($p<0.01$) and 28.2 ± 9.75 % ($p<0.01$) of each corresponding control, respectively.

Effect of adenosine on CAs secretion evoked by MCP

Since the results obtained in figure 3, 4, 5 and

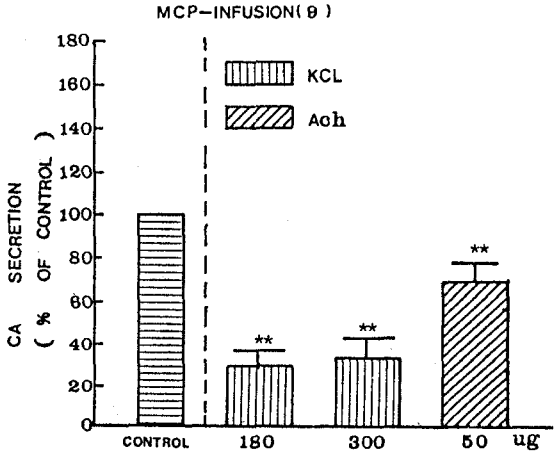

Fig. 6. The effect of infusion of metoclopramide on secretion of catecholamines evoked by potassium chloride and Ach. KCl (180 and 300 ug) and Ach (50 ug) were given into perfusion stream after perfusion of Krebs containing MCP (200 ug/30 min) for 30 min. Other legends and methods are the same as in Fig. 2 and 3.
**: $p<0.01$

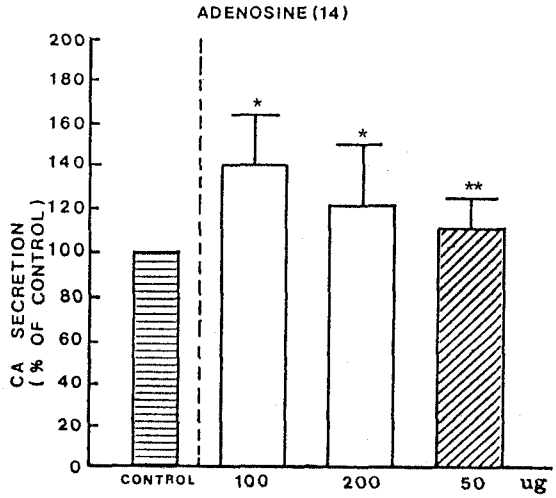

Fig. 7. The effect of adenosine on secretion of catecholamines evoked by MCP or Ach. The adrenal gland was perfused for 30 min with Krebs solution containing 0.18 mM-adenosine and in the presence of adenosine secretion of catecholamines was produced by MCP or Ach. Other legends and methods are as in Fig. 2 and 3.
*: $p<0.05$, **: $p<0.01$

6 indicate that CA secretion evoked by MCP are produced primarily by the action of Ach released via stimulation of nicotinic and nuscarinic receptors located on chromaffin cells. Therefore, it was of particular interest to study the effect of adenosine on CA secretion evoked by MCP and that evoked by exogenous Ach. The results of these experiments are shown in figure 7. Perfusion of the adrenal gland with 0.18 mM-adenosine for 30 min resulted in increment in CA secretion evoked by 100 ug and 200 ug-MCP to $138.5\pm48.18\%$ ($p<0.05$) and 120.2 ± 30.0 ($p<0.05$) of each control response, respectively. Ach-evoked CA output was also increased to $113.3\pm15.8\%$ ($p<0.01$) of the control.

Effect of ouabain on CA secretion induced by MCP

Since ouabain is known to inhibit sodium-pump in several test systems (Schwartz, 1976; Akera, 1977), and since it has been demonstrated that cardiac glycosides cause the release of CA in the perfused bovine adrenal gland (Banks, 1967), guinea pig vas deferens (Ozawa and Katsuragi, 1974), rabbit heart (Lindmar and Loffelholz, 1974), cat spleen slices (Garcia and Kirpekar, 1973 b) and the perfused cat adrenal gland (Garcia *et al.*, 1980), it was decided to investigate the effect of ouabain on CA secretion evoked by MCP or Ach in the rat adrenal gland. In 20 rat adrenal glands, after obtaining the control secretion the adrenal gland was perfused with 2 uM-ouabain for 30 min and secretory response was evoked in the presence of ouabain. As shown in Figure 8, ouabain enhanced greatly secretion of CA evoked by 100 ug and 200 ug-MCP to 136.7 ± 19.8 ($p<0.05$) and $129.02\pm17.34\%$ ($p<0.01$) of each corresponding control, respectively. Ach-evoked CA secretion was also potentiated significantly to $119.6\pm12.55\%$ ($p<0.05$) of the control value.

Effect of perfusion with Ca^{++}-free medium plus EGTA on CA secretion evoked by MCP

Since the physiological release of CA and dopamine-beta-hydroxylase from the perfused cat adrenal gland is dependent on the extracellular calcium concentration (Dixon, Garcia and Kirpe-

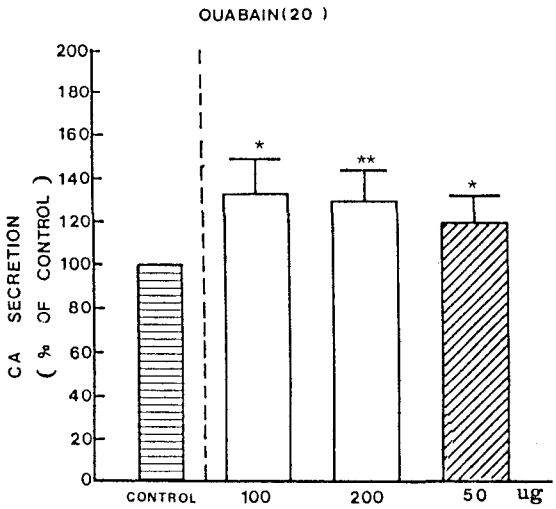

Fig. 8. The effect of ouabain on secretion of catecholamines evoked by MCP or Ach. Catecholamines-secretion by MCP was induced 30 min after beginning of Krebs solution containing 2 uM-ouabain. Other legends and methods are as in Fig. 2 and 3.
*: $p<0.05$ **: $p<0.01$

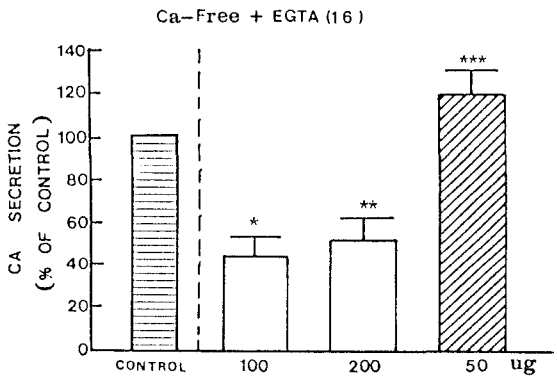

Fig. 9. The effect of perfusion with Ca-free medium and EGTA on catecholamine-secretion evoked by MCP or Ach. After obtaining control responses the gland was perfused with Krebs solution containing 5 mM-EGTA without calcium for 30 min and then stimulated with MCP or Ach. Other legends are the same as in fig. 2 and 3.
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

kar, 1975), it is of particular interest to test whether the secretory effect induced by MCP in this preparation is also related to extracellular calcium ions. Thus, the adrenal glands were perfused with calcium-free Krebs solution containing 5 mM-EGTA for 30 min after obtaining the control responses. The results of this experiment are shown in figure 9. In 16 rat adrenal glands, the perfusion of this solution led to clearly reduction in CA secretion evoked by 100 ug and 200 ug-MCP by $54.9 \pm 18.93\%$ ($p<0.05$) and $48.79 \pm 13.56\%$ ($p<0.01$) of the corresponding control, respectively. But Ach-induced CA secretion was rather enhanced to $119.1 \pm 11.15\%$ ($p<0.001$) of the control value.

DISCUSSION

The present experimental findings show that MCP evokes significantly the release of CA from the isolated perfused rat adrenal gland by a calcium-dependent exocytotic mechanism, and that the secretory effect of CA induced by MCP may be partly due to activation of cholinergic muscarinic receptors located in the adrenal gland rather than nicotinic receptors and partly to the direct action.

The adrenal medulla has been employed as a model system to study numerous cellular functions involving not only noradrenergic nerve cells but also neurons in general. One of such functions is neurosecretion. During the neurogenic stimulation of the adrenal medulla, Ach is released from the splanchnic nerve endings and activated cholinergic receptors on the chromaffin cell membrane (Viveros, 1975). This activation initiates a series of events known as stimulus-secretion coupling, culminating in the exocytotic release of CA and other components of the secretory vesicles into the extracellular space. In general, two mechanisms are involved in the secretion of adrenal medullary hormones. Upon excitation of splanchnic nerves, Ach is released from the nerve terminals, and then it activates nicotinic and muscarinic receptors of the chromaffin cells, causing exocytotic secretion of CA.

In the present study, the fact that MCP caused the secretory effect of CA from the rat adrenal gland demonstrated clearly that MCP may produce hypertensive crisis especially in patients with pheochromocytoma as shown in previous reports (Plouin *et al.*, 1976; Agabiti-Rosei, *et al.*, 1977; Abe *et al.*, 1984).

Ach, the physiological presynaptic transmitter

at the adrenal medulla, releases CA and dopamine-beta-hydrxylase by a calcium-dependent secretory process (Viveros *et al.,* 1968; Dixon *et al.,* 1975). Since MCP also provokes cholinergic properties especially in gastrointestinal smooth muscles of various species (McCallum *et al.,* ; Stadaas and Aune, 1971; Hay and Man, 1979; Hay, 1977), a question arises whether secretion of CA evoked by MCP in the rat adrenal gland is due secondarily to release of Ach from cholinergic terminals present in gland or the the direct action on adrenal medulla.

Since chlorisondamine, a well-known ganglionic blocking agent (Gilman *et al.,* 1985), did not block completely the secretory responses of MCP, it is felt that MCP effect may be due partly to stimulation of muscarinic receptors or to the direct action on the chromaffin cells in addition to nicotinic action. In the present work, in view of the fact that pretreatment with atropine inhibited greatly the CA secretion of MCP, MCP seems to have greater activity to muscarinic Ach receptors than to nicotinic receptors in the secretory effect of CA. In previous studies, it has been found that atropine reduces of abolishes actions of MCP on gastrointestinal smooth muscles (Eisner, 1968; Kowalewski and Kalode; Beani *et al.,* 1970; Fox and Behar, 1980). However, inhibition of acetylcholinesterase with physostigmine (Gilman *et al.,* 1985) before the MCP injection clearly enhanced the drug's effect. This knotty contradictory finding can be explained if MCP also releases Ach from presynapyic cholinergic sites, as it does in other cholinergic systems such as gastrointestinal smooth muscle but the amounts of Ach being released are probably low and the Ach is quickly degraded by AchE before reaching the muscarinic and nicotinic receptors located on the surface of the chromaffin cell. Moreover, the finding that during perfusion of MCP Ach-evoked secretion was markedly reduced may support the potentiating effect of physostigmine to CA secretion evokedby MCP. The fact that nicotinic (but not muscarinic) stimulation also releases soluble Ach from the chromaffin cells by a calcium-dependent mechanism (Mizobe and Livett, 1981; 1983) appears to be considerably related to these experimental results. Despite of a series of these facts, it is not likely to exclude that MCP may have at least partly the direct action in CA secretion. Any way, these observations strongly sugest that MCP may produce the release of CA through the activation of muscarinic receptors as well as nicotinic receptors.

Douglas *et al.,* (1967) have shown that muscarine may activate voltage-dependent calcium permeability to promote secretion. In the isolated chromaffin cells of the gerbil, the depolarizing effect of pilocarpine is blocked by atropine alone; depolarizing effect of Ach is only partially blocked when hexamethonium is added alone, but completely blocked when atropine and hexamethonium are added together. Furthermore, Brandt and his collegues (1976) also reported that atropine blocked the depolarizing effect of Ach in the rat adrenal chromaffin cells.

Adenosine perfusion caused significant enhancement of CA secretion evoked by both exogenous Ach and MCP. Generally, it is known that adenosine inhibits norepinephrine release from sympathetic neurons as well as acetylcholine release at the neuromuscular junction and ganglia (Fredholm and Hedqvist, 1980), and that in the brain, adenosine is also almost uniformly inhibitory in its action of neuronal firing (Phills and Wu, 1981). Wakade and Wakade (1979;1981) have also reported that splanchnic nerve terminals are capable of generating action potentials upon electrical excitation, and adenosine interferes with secretory process by shortening the duration of nerve action potential and thereby reducing calcium influx. Adensosine thus could reduce Ach release, and thereby CA secretion.

In contrast to these reports, adenosine potentiated markedly CA secretion induced by MCP or Ach in the present experiment. In our previous study, it was found that adenosine enhances Ach-evoked secretory activity of CA in rabbits, and that this effect was brought through adenosine receptors located on chromaffin cells (Lim and Choi, 1986). MCP-evoked secretory effect of CA was potentiated by adenosine-treatment in the same manner as to that of Ach, and it seems that the effect of MCP may involve adenosine receptors.

In addition to this effect of MCP to adenosine treatment, we found that adenosine also enhanced Ach-induced CA secretion which is evidently contrary to the findings of Wakade (1981), although the reason why it is so is not clear.

The indispensible role of calcium in the neurosecretory process has been thoroughly established. According to the assumptions of Baker and Knight (1978; 1980), the relationship between concentration of intracellular calcium and transmitter release has not yet been determined in nerve

terminals. As mentioned above, calcium plays the crucial role in parallels between depolarization-neurotransmitter release coupling in many other types of secretory cells (Douglas, 1968; Schulz and Stolze, 1980; Williams, 1980). In the present work, removal of extracellular Ca^{++} depressed CA secretion evoked by MCP or Ach. The secretory response evoked by Ach was almost extinguished in calcium-free Kdrebs solution containing 5 mM-EGTA, while that evoked by MCP was maintained at the level of about 52% of the control secretion in zero Ca^{++} medium. It has been shown that CA outflow in response to Ach are almost blocked by the exposure of Ca^{++}-free medium in various animal adrenal glands (Douglas and Rubin, 1963; Philliphu and Schmann, 1962; Oka *et al.,* 1965; Kanno, 1978).

In this experiment, the reason for the considerable response to MCP in Ca^{++}-free Krebs solution is not clear. It may be that chromaffin cells of the rat adrenal gland contain an intracellular store of calcium which participates in the secretion of CA as shown in the bovine adrenal gland (Baker and Knight, 1978). Such a store may not be easily depleted by removal of extracellualr calcium. Some investigators (Bozler, 1968; Ohashi *et al.,* 1974; Casteels and Raeymaekers, 1979) reported that intracellular stores of calcium have been shown to play some role in contraction of smooth muscle produced by noradrenaline or Ach in Ca^{2+}-free medium. Since MCP promotes the release of CA by extracellular calcium-dependent process, the underlying secretory mechansim seems to be similar to the physiological exocytotic mechanism. It therefore seems probable that the action of MCP is achieved by a rise in the intracellular ionized calcium concentration. In support of such an idea, recently, Kao and Schneider (1985) found that Ach evoked a large increase in cytosolic free-calcium in bovine chromaffin cells, most of which is blocked by hexamethonium treatment or removal of extracellular calcium, and that a small component of the Ach-evoked rise in cytosolic free Ca^{++} is independent on extracellular and is unaffected by atropine. These results suggested that muscarinic receptors regulate cytosolic calcium in chromaffin cells by a new mechanism different from that of nicotinic receptors, a mechanism utilizing an intracellular calcium source.

In ther present study, ouabain enhanced CA secretion evoked by Ach or by MCP from the rat adrenal gland. Since ouabain facilitated CA secretion evoked by exogenous Ach, it appears that effect of ouabain is mostly on the postsynaptic sites or the chromaffin cells of the adrenal gland. It is well-known that ouabain is a specific inhibitor of Na, K-activated ATPase in many biological systems (Schwartz, 1976; Akera, 1977).

Garcia *et al.,* (1980) showed that ouabain releases CA from the perfused cat adrenal gland by a calcium-dependent exocytotic mechanism, which is due to a direct action on chromaffin cell itself, and that this secretory effect of CA evoked by ouabain is exerted through redistribution of monovalnt cations secondary to the inhibition by glycoside of the sodium pump.

It this is the case, it may be that inhibition of the sodium pump by MCP as in the case of ouabain will ultmately lead to intracellular sodium accumulation and potassium loss, and that such monovalent cation redistribution may cause a rise of intracellular ionized calcium level first, by depolarization of chromaffin cells or secretion, and by activation of the sodium-depenadent calcium influx system. Wakade (1981) has shown that the mechanism responsible for producing the facilitation by ouabain is believed to be very similar to inhibition of Na, K-ATPase for the enhancement of Ach release from presynaptic nerves in K^+-free medium. Furthermore, in terms of the fact that MCP-perfusion depressed significantly the secretory effect of CA evoked by Ach in the present experiment, it is thought that MCP may cause CA secretion by the direct mechanism similar to ouabain.

It is well established that depolarization of the chromaffin cell produces an increase in calcium uptake (Douglas and Poisner, 1962), but it has also been shown that maintained depolarization of adrenergic neurons and chromaffin cells induces a sharp secretory response which rapidly dsensitizes, probably because inactivation of a membrane calcium channel follows a brief period of activation (Baker and Rink, 1975; Garcia *et al.,* 1976).

In the present experiment, The secretion of CA evoked by MCP was a short-acting one (data not shown) and this fact was dissimilar to that induced by ouabain (Garcia *et al.,* 1980), which is a long-lasting and does not apparently desensitize.

From the above discussions, we found that there is much differences in the mechanism of secretory effect of CA between the present work and the study by Abe *et al.,* (1984), in which MCP-induced hypertensive crisis in patient with pheochromocytoma is due to attribution of CA release by presynaptic dopaminergic blocking

effects. Anyway, these experimental findings suggest that MCP could be potentially dangerous if used indiscriminately for patient with undiagnosed pheochromocytoma or with hypertension, and therefore that this drug should be avoided in those patients.

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= 국문초록 =

흰쥐 적출 부신에서 Metoclopramide의 Catecholamine 분비작용에 관한 연구

조선대학교 의과대학 약리학교실

임동윤 · 김규형 · 최철희 · 유호진 · 최동준 · 이은화

Metoclopramide (MCP)는 역류성식도염, 위하수증, 항암제에 의한 구토 증상을 치료하는데 사용되고 있는 dopamine 수용체 차단제로 알려져 있다. 본 연구에서는 흰쥐의 적출 관류부신에서 catecholamine (CA)의 분비작용에 미치는 영향을 검토하여 다음과 같은 결과를 얻었다.

MCP는 부신정맥내로 주입하였을때 용량의존적으로 CA 분비작용을 나타냈다. 이러한 MCP의 CA 분비작용은 atropine 전처치로 차단되었으나 chlorisondamine 처치에 의해서는 완전 차단되지 못하였다.

MCP 분비작용은 physostigmine, adenosine, ouabain의 전처치로 현저히 증강되었다. 그러나 5 mM-EGTA 함유 Ca^{++}-free Krebs 액으로 관류하였을때 MCP의 CA 분비작용은 현저히 억제되었다. MCP (200 ug/30 min)를 관류한 실험에서는 KCl이나 acetylcholine의 CA 분비작용이 유의있게 감소되었다. 이상의 실험결과로 보아 MCP는 칼슘의존적기전에 의해 CA를 유리 시키며, 이러한 분비작용은 부신에서 nicotine 수용체 보다도 muscarine 수용체의 활성화에 더 기인 되는 것으로 생각되며, chromaffin cell에 대한 일분 직접작용도 개재되어 나타나는 것으로 사료된다.