Korean J. of Pharmaclolgy
Vol. 27, No. 1, pp. 53-67, 1991

Studies on Secretion of Catecholamines Evoked By DMPP and McN-A-343 in the Rat Adrenal Gland

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ABSTRACT

The characteristics and differences between DMPP and McN-A-343 on the secretory effect of catecholamines(CA) were studied in the isolated perfused rat adrenal glands. DMPP(100 uM) and McN-A-343(100 uM) perfused into an adrenal vein of the gland casued significant increases in CA secretion. On molar basis the secretory effect of McN-A-343 was about one fifth as potent as that of DMPP. Tachyphylaxis to releasing effects of CA evoked by DMPP and McN-A-343 was not observed by repeated perfusion of these agents.

The DMPP-evoked CA secretion was significantly inhibited by pretreatment with chlorisondamine, desipramine and prefusion of Ca^{2+}-free Krebs solution containing EGTA, while it was not affected by pirenzepine, ouabain and physostigmine. However, pretreatment with atropine rather enhanced CA release by DMPP.

The releasing effect of CA induced by McN-A-343 was markedly depressed by pretreatment with atropine, pirenzepine, chlorisondamine, physostigmine, and perfusion of Ca^{2+}-free medium plus EGTA but was not influenced by desipramine, except for the case of ouabain which clearly potentiated CA release by McN-A-343.

These experimental results suggest that both DMPP and McN-A-343 cause greatly secretion of CA from the isolated perfused rat adrenal glands by a calcium-dependent exocytotic mechanism. The secretory effect of DMPP is due to the stimulation of cholinergic nicotinic receptors and the secretion by McN-A-343 via activation of selecive M_1-muscarinic receptors in the adrenal gland. It is also thought that the DMPP-evoked secretory effect is much greater than McN-A-343-induced effect.

Key Words: DMPP, McN-A-343, Adrenal gland, Catecholamine-secretion

It has been known that in genral two mechanisms are involved in the secretion of adrenal medullary hormones. Upon excitation of splanchnic nerves, acetylcholine(Ach) is released from the nerve terminals, which then activates nicotinic and muscarinic receptors of the chromaffin cells, causing exocytotic secretion of catecholamines(CA).

Dimethylphenylpiperazinium(DMPP) is a synthetic quaternary ammonium compound that is more selective fro ganglionic receptors as a typical autonomic ganglionic stimulant(Rang and Dale, 1987). It also causes the hypertensive responses mediated by nicotinic receptors in cats, dogs and rats, which disappear after by the blockade of adrenergic receptors and the autonomic ganglia(Chen *et al.*, 1951).

The release of epinephrine from the adrenal medulla in response to splanchnic nerve stimulation or nicotinic agonists is mediated by activation of nicotinic receptors located on the chromaffin cells. The exocytotic CA release

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from chromaffin cells appears to be essentially similar to that occuring in noradrenaergic axons(Douglas, 1986; Sorimachi and Yoshida, 1979).

It has also been shown that the chromaffin cells of the rat adrenal medulla contain muscarinic receptors which are linked to the secretion of CA(Wakade and Wakade, 1983). McN-A-343 (3-(m-chlorophenyl-carbamoyloxy)-2-butynyl trimethyl ammonium chloride)) increases the blood pressure and heart rate, both responses being readily blocked by atropine(Roszkowsk, 1961; Fozard and Muscholl, 1972). This unusual combination of effects of a muscarinic agonist has been explained by a selecetive stimulatory muscarinic effect on sympathetic ganglia which overcomes the much weaker direct muscarinic effects on the heart and vasculature. This pressor response is thought to be mediated through the M_1subtype(Hammer and Giachetti, 1982).

However, recently, the use of McN-A-343 as a selective M_1receptor agaonist has been questioned on the ground that its selectivity may arise from differences in receptor reserve in various tissuse(Eglen *et al.*, 1985).

Futhermore, more recently, Wakade and his collegues(1986) have reported that McN-A-343 and oxotremorine appear to act as partial agonists. Of their particular interest was the finding that McN-A-343 exerted antinicotinic as well as antimuscarinic effects on the secretion of CA from the rat chromaffin cells. Since, there is a clear controversy about the role of the muscarinic receptors in the secretion of adrenomedullary hormones along with the nicotinic receptor, it is of particular interest to recharacterize the nature of the muscarinic receptors and nicotinic receptors in the secretory process. For this purpose differences in CA secretion from the rat adrenal gland by using McN-A-343 and DMPP as selecitve M_1muscarinic and nicotinic agonists, respectively were investigated in the present study.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague Dawley rats, weighing 180-300g, were anesthetize with ether. The adrenal gland was isolated by the method 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 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 cannulations.

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 branches of adrenal vein, 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 cannulation. A small slit was made into the adrenal cortex just opposite the entrance of adrenal vein. Perfusion of the gland was started, making it sure that no leakage was present, and the perfusion fluid escaped only from slit made in adrenal cortex. Then the adrenal gland, along with the ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37 ± 1°C(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-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 constantly bubbled with 95% O_2± 5% CO_2, and the final pH was adjusted to 7.4 ± 0.5. 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 1 minutes or a single injection of Ach(50 ug) in a volume of 0.05 ml were made into the perfusion stream via a three way stopcock(Fig.1).

In the preliminary experiments it was found that upon administration of the above doses of drugs secretory response to Ach returned to

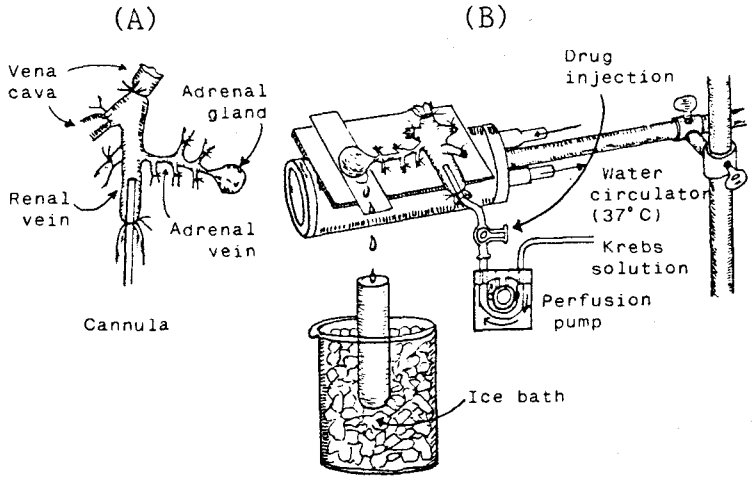

Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamine in the isolated perfused adreanal gland of the rat.

preinjection level in about 4 min, but the responses to DMPP and McN-A-343 in 6~9 min. Therefore, each sample to Ach was collected for 4 min, samples to DMPP and McN-A-343 6~9 min, after administration of the drugs. Generally, the adrenal glands were perfused with normal Krebs solution for about one hour before drug administration. The adrenal perfusate was collected in chilled tubes. Details of the collection of samples are given in Result's section.

Collection of perfusate

As a rule, prior to each stimulation with cholinergic agonists perfusates were collected(4 to 9 min) to determine the spontaneous secretion of CA("background sample"). Immediately after the collection of the "background sample", collection of the perfusate 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 9 min. The amounts secreted in the "background sample" have been substracted from those secreted from the "stimulated sample" to obtain net secretion of CA, which is shown in all of the figures.

To study the effects of a test agent on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing the agent for bout 20~30 min, then the perfusate was collected for a specific time period("background sample"), and then the medium was changed to the one containing the test agent plus the stimulating agent and the perusates were collected for the same period as that for the "background sample".

Measureement of catecholamines

CA content of perfusate was measured directly by the fluorometirc method of Anton and Sayer (1962) without the intermediate purification alumina, for the reasons described earlier (Wakade, 1981), using fluorospectrophotmeter (Shimazu Co.). A volume of 0.2 ml of the perefusate was used for the reaction. The CA content in the perfusate of stimulated glands by Ach or DMPP or McN-A-343 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 were presented as means with their standard errors, and the significance of differences was anlyzed using Student's t-test.

Drugs and their sources

The follwoing drugs were used: acetylcholine

chloride, 1.1-dimethyl-4-phenyl pierazinium iodide(DMPP), norepinephrine bitartrate, physostigmine sulfate, ouabain octahydrate, disodium EGTA, desipramine hydrochloride(Sigma Chemical Co., U.S.A), pirenzepine. 2 HCL(Shin-Poong Pharmaceutical Manuf, Korea), chlorisondamine chloride(Ciba Co., U.S.A). and atropine sulfate(Merk, Germany). Drugs were dissolved in distilled water(Stock) and added to the Krebs solution as required. All drug concentrations are expressed in terms of molar base except the case of Ach.

RESULTS

Catecholamine secretions induced by Ach, DMPP and McN-A-343

The resting CA secretion from the perfused rat adrenal glands attained a steady level 60 min after the start of perfusion with Krebs solution. When Ach(50 ug) was injected into the perfusion stream via a three way stopcock, CA secretion was 334.2 ± 29.00 ng($P<0.001$) for 4 min from 60 rat adrenal glands.

Perfusion of adrenal gland with selective nicotinic receptor agonist, DMPP(100 uM) and a selective M_1-muscarinic agonist, McN-A-343 (100 uM) for 2 min periods resulted in rapid and marked increases in secretion of CA that was maximal for the fraction collected during the first 3 min period of DMPP or McN-A-343 perfusion declinging rapidly to the pre-DMPP or McN-A-343 level during the subsequent 6 to 9 min periods, as shown in figure 2 and table 1. The secretions evoked for the first period(0~3 ming) after 2 min perfusion of DMPP and McN-A-343 were 335.6 ± 16.46 ng($P<0.001$, $n=42$) and 74.3 ± 5.77 ng($P<0.001$, $n=48$) respectively. The secretion of the second period(3~6 min) to DMPP and McN-A-343 were 118.3 ± 7.22 ng/3 min($P<0.001$, $n=42$) and 2.4 ± 0.98 ng/3 min (NS, $n=48$), respectively, and those of the third period(6~9 min) after perfusion of drugs were 28.7 ± 5.61 ng/3min($P<0.001$) only in the case of DMPP perfusion without any secretion evoked by McN-A-343. From our unpublished resultes, it was shown that perfusion with lower drug concentrations did not evoke the secretion of CA by DMPP(1~10 uM) and McN-A-343(1~30 uM). A further increase to very high concentration(200~1000 uM) of these drugs caused only a modest secretion in perfusion of McN-A-343, but much greater increase in the case pf DMPP. Therefore, in all subsequent experiments a concentraion of 100 uM of those durgs was used with a dose of 50 ug Ach in order to compare with their results.

Figure 3 and 4 show time course and effects of repeated administration of DMPP and McN-A-343. As shown in Figures, when DMPP and McN-A-343 were perfused into the perfusion stream of the adrenal glands three times consecutively at 30 min intervals, respectively, there was no difference between each periods after perfusion of both drugs.

Table 1. Secretion of catecholamines evoked by DMPP and McN-A-343 from the isolated perfused rat adrenal gland

Type of stimlus to evoke secretion	Dosage of administration	Time of collection(min)	Secretion of Catecholamine	Number of animal
Acetylcholine	50 ug	0~4	334.2 ± 29.00	62
DMPP	100 uM	0~3	335.6 ± 16.46	42
		3~6	118.3 ± 7.22	42
		6~9	28.7 ± 5.61	42
McN-A-343	100 uM	0~3	74.3 ± 5.77	48
		3~6	2.4 ± 0.89	48

Secretion of CA are expressed with mean ± S.E. of differences between pre-and post-stimulation. The adrenal glands are stimulated with perfusion of DMPP(100 uM) or McN-A-343(100 uM) for 2 min, or with single injection of acetylocholine(50 ug). The perfusate to acetylcholine was collected for 4 min, but DMPP and McN-A-343 for 6 ~9 min at 3 min intervals.

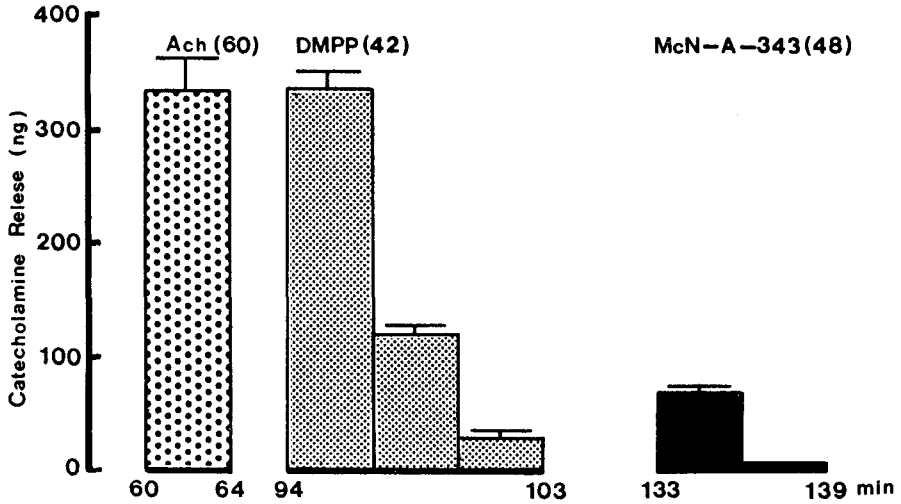

Fig. 2. The time course of catecholamine secretion evoked by perfusion with acetylcholine(Ach), DMPP and McN-A-343. Secretion of catecholamines(CA) was evoked by Krebs solution by introducing injection of Ach(50 ug), perfusion of DMPP(100 uM) and McN-A-343 for 2 min into the perfusion stream respectively. 60 min after the beginning of perfusion with Krebs solution, following the injection of drugs, the perfusate was collected for 4~9 min. Vertical bars represent S.E. of the mean. Numerals in the upper bracket indicate number of experimental animal.
Ordinate: the amounts of catecholamine secreted from the adrenal gland. Abscissa: time of the secretory effect evoked by each agent.

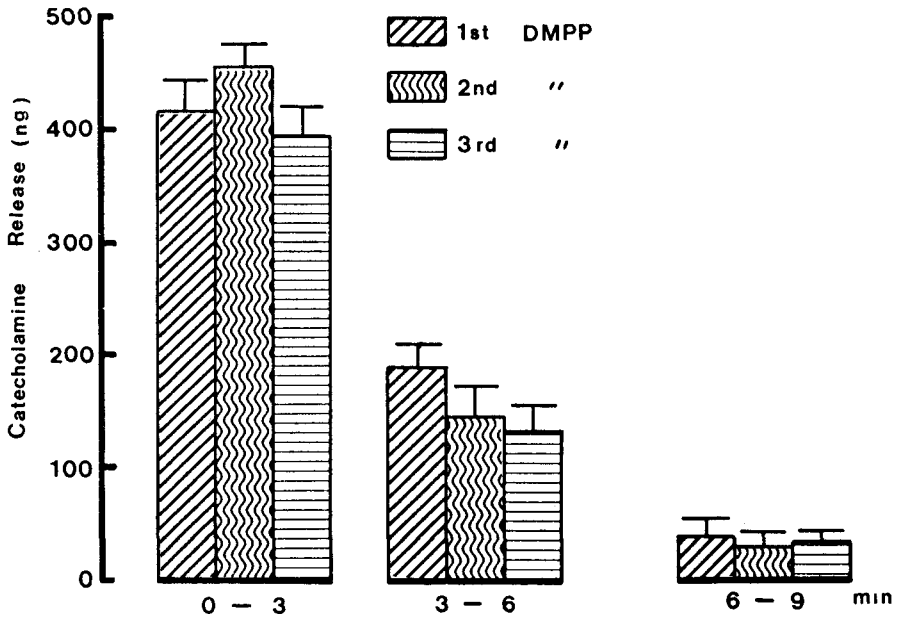

Fig. 3. Effects of repetitive perfusion of DMPP on secretion of catecholamines from the isolated perfused rat adrenal gland. DMPP(100 uM) was perfused repeatedly into the perfusion stream for 2 min at 30 minute intervals 60 min after beginning perfusion of normal Krebs solution. No signficance between periods of DMPP-perfusion was obtained from 7 rat adrenal glands. Other legends are the same as in fig. 2.

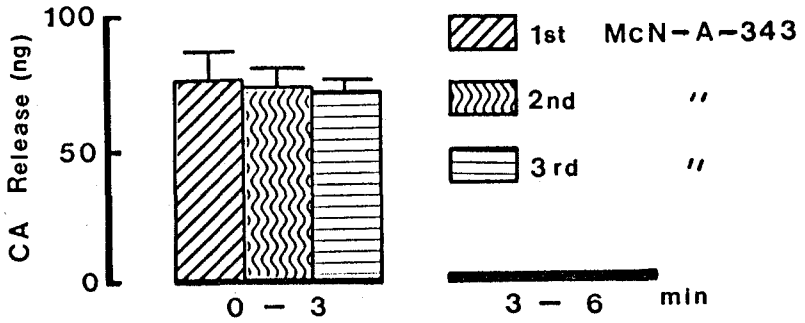

Fig. 4. Effects of repetitive perfusion of McN-A-343 on secretion of catecholamines from the rat adrenal gland. 100 uM McN-A-343 was perfused into the perfusion stream repeatedly at 30 min intervals. There was no significance between periods of McN-A-343 perfusion. Other legends and methods are the same as in fig. 2.

The effects of atropine and pirenzepine on the secretion of CAs evoked by DMPP or McN-A-343

It is known that both atropine and pirenzepine are muscarinic antagonist and pirenzepine is also more selectve M_1-muscarinic antagonist (Doods *et al.*, 1987; Hammer *et al.*, 1987). Thus, it would be very interesting to test the influence of atropine or pirenzepine on CA secretion evoked by DMPP or McN-A-343.

In the present study, the secretory effects of DMPP or McN-A-343 were evoked in the adrenal gland pretreated with 2.25×10^{-6} M-atropine or 2×10^{-6} M-pirenzepine for 30 min, respectively. In 6 rats, 100 uM-DMPP-induced secretory effect of CA in the presence of atropine was greatly increased to $141.8\pm16.6\%$($P<0.001$) of the corresponding control value(100%) while 100 uM-McN-A-343-evoked secretion of CA was clearly depressed by $21.9\pm2.85\%$($P<0.001$) of the control group as shown in figure 5. However, after pretreatment with 2×10^{-6} M pirenzepine, McN-A-343-induced secretion was significantly reduced by $7.1\pm0.44\%$($P<0.001$) of the corresponding control values, while DMPP-induced CA secretion was tendency to reduce by $86.4\pm9.25\%$(NS, n=4) of the control. Figure 5 shows the effect of pirenzepine on secretion of CA evoked by DMPP or McN-A-343. Ach-evoked CA secretion was greatly inhibited by pretreatment with both atropine and pirenzepine as shown in figure 5 and 6.

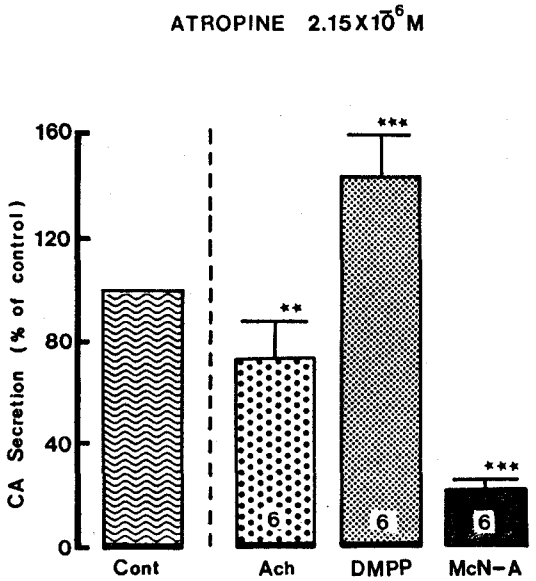

Fig. 5. The effect of atropine on DMPP-and McN-A-343-evoked secretion of of catecholamines (CA). Atropine was present 30 min before perfusion of DMPP or McN-A-343. The histogram represent the mean values of DMPP- or McN-A-343-induced secretion of catecholamines expressed as percentages of both agents-induced secretion as the control(100 %) before atropine-treatment. The number of experiments is shown inside each histogram. The asterisks denote significant reduction or increase from the corresponding control (Cont.) **: $P<0.01$, ***: $P<0.001$.

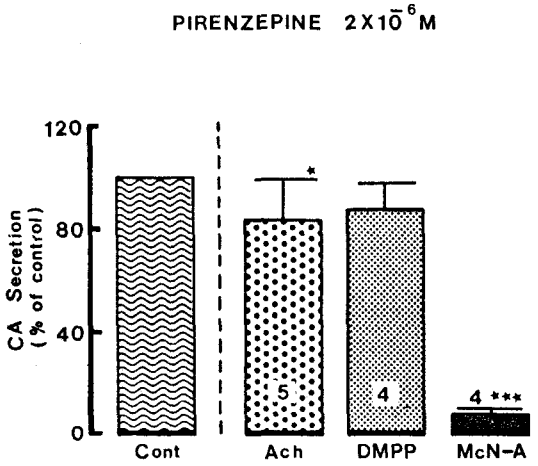

Fig. 6. The effect of pirenzepine on secretion of catecholamines(CA) evoked by DMPP or McN-A-343. Pirenzepine was present 20 min before perfusion of DMPP or McN-A-343. Other legends and the method are the same as in fig. 4. * : $P>0.05$, ***: $P<0.001$.

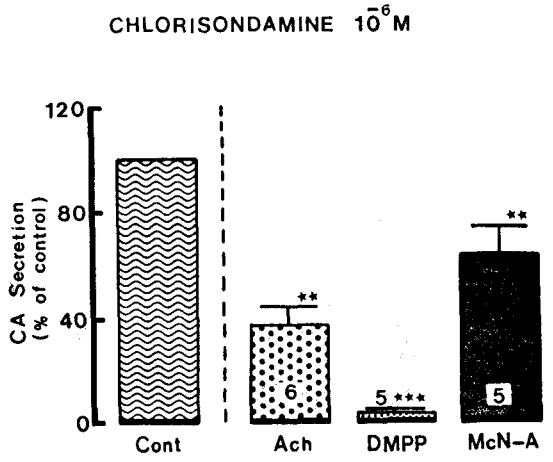

Fig. 7. The effect of chlorisondamines on secretion of catecholamines(CA) evoked by DMPP or McN-A-343. Other legends and the method are the same as in fig. 4 and 5
**: $P<0.01$, ***: $P<0.001$.

The effect of chlorisondamine on the release of CAs evoked by DMPP or McN-A-343

In order to observe the effect of chlorisondamine, a selective nicotinic receptor antagonist (Gliman *et al.*, 1991), on CA secretion evoked by DMPP or McN-A-343, the adrenal gland was perfused with chlorisondamine(10^{-6} M) for 20 min before the introduction of DMPP or McN-A-343.

In the presence of chlorisondamine, the CA releases evoked by 100 uM - DMPP, 100 uM-McN-A-343 and 50 ug-Ach were markedly depressed by $3.5 \pm 0.36\%$($P < 0.001$, $n = 5$), $63.6 \pm 10.30\%$($P<0.01$), $n=5$) and $36.8 \pm 6.53\%$($P<0.01$, $n=6$) of the each corresponding control values, respectively. Figure 7 represents the inhibitory effect of chlorisondamine on CA releases induced by DMPP or McN-A-343.

The effect of ouabain on the release of CAs evoked by DMPP or McN-A-343

Since it has been reported that cardiac glycosides increase both spontaneous and evoked CA secretions from perfused adrenal gland(Banks, 1967; 1970; Garcia *et al.*, 1981b; Wakade, 1981; Nakazato *et al.*, 1986) and isolated adrenal chromaffin cells(Aunis and Garcia, 1981; Sorimachi *et al.*, 1981; Pocock, 1983a,b), it is likely of particular interest to investigate the effect of ouabain on CA secretion evoked by DMPP or McN-A-343. As shown in figure 8, after perfusion of ouabain(5×10^{-7} M) for 30 min CA releases of McN-A-343(100 uM) and Ach(50 uM) were clearly enhanced to $149.7 \pm 24.03\%$($P < 0.01$, $n=6$) and $118.10 \pm 4.78\%$($P<0.001$, $n=10$) of each control response, respectively, while secretory effect of DMPP(100 uM) was almost the same to the control value($101.01 \pm 13.\%$, $n=16$, Ns).

The effect of Physostigmine on the release of CAs evoked by DMPP or McN-A-343

In the light of the facts that DMPP-and McN-A-343-induced CA secretions were significantly depressed by pretreatment with atropine, pirenzepine and chlorisondamine and potentiated by ouabain as shown in figure 5,6,7 and 8, it was decided to examine the effect of physostigmine, and anticholinesterase(Gilman et al., 1991), on secretory effects of CA evoked by DMPP and McN-A-343 in the isolated perfused rat adrenal glands.

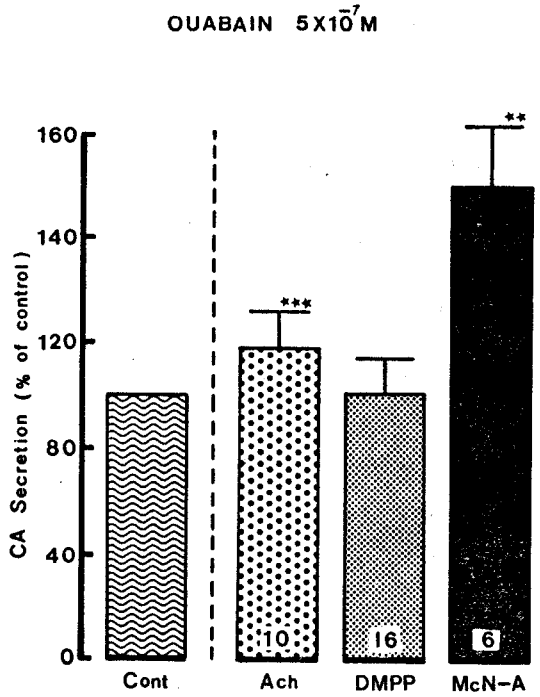

Fig. 8. The effect of ouabain on catecholamines secretion evoked by DMPP or McN-A-343. Ouabain was present 20 min before perfusion of DMPP or McN-A- 343. Other legends and the method are the same as in fig. 4 and 5. **: P<0.01, ***: P<0.001.

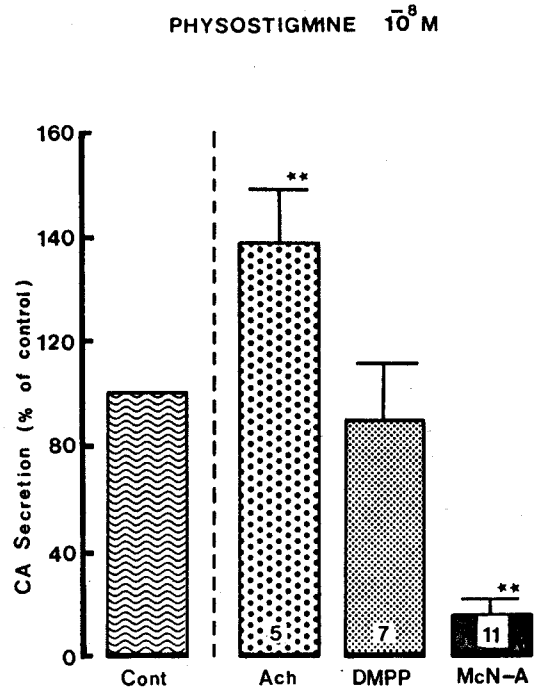

Fig. 9. The effect of physostimgmine on catecholamine-secretion evoked by DMPP or McN-A-343. Physostigmine was present 20 min before perfusion of DMPP or McN-A-343. Other legends and the method are as in fig. 4 and 5. ** : P<0.01.

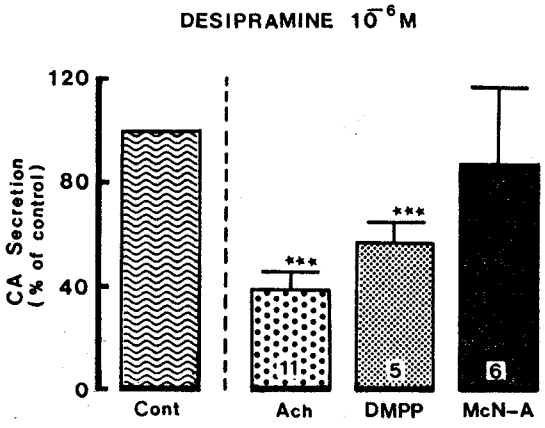

Fig. 10. Effect of desipramine on secretion of catecholamnes(CA) evoked by DMPP or McN-A-343. Desipramine was present 20 min before perfusion of DMPP or McN-A-343. Other legends and the method are as in fig. 4. and 5. ***: P<0.001.

The glands were perfused with prior to perfusion of drugs for 30 min. CA secretory effect evoked by DMPP(100 uM) in the presence of physostigmine was tendency to reduce by 88.1± 19.69%(ns, n=7) of the corresponding control response. However, CA secretion by McN-A-343 was markedly attenuated by 16.9±6.97%(P<0.01, n=11) of the control while Ach-evoked CA secretion was greatly potentiated to 136.8±21.525 (P<0.01, n=5) of the corresponding control response(Fig. 9).

The effect of desipramine on CAs release evoked by DMPP or McN-A-343

It has been found that the inhibitors of the neuronal membrane transport of CA, desipramine and cocaine, reduce nicotine-evoked norepinephrine release from sympathetic nerves (Su and Bevan, 1970; Westfall and Brasted, 1972;

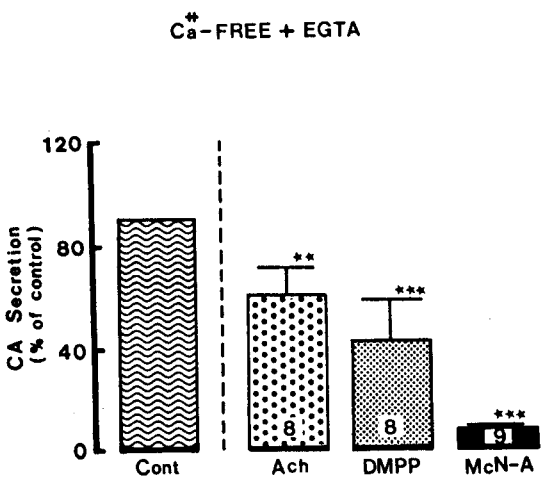

Fig. 11. The effect of perfusion of Ca^{2+}-free-medium plus EGTA on catecholamine(CA) secretion evoked by DMPP or McN-A-343. Ca^{2+}-free Krebs solution containing 5 mM EGTA was perfused 30 min before perfusion of DMPP or McN-A-343. Other legends and the method are the same as in fig. 4. and 5.
**:$P<0.001$.

1974; Wakade and Wakade, 1984; Collect and Story, 1984). Therefore, it was very exciting to observe the effect of desipramine on secretory effect of DMPP and McN-A-343. Rat adrenal glands were perfused with desipramine(10^{-6} M) for 30 min before stimulation with agents. Ach- and DMPP-induced secretory effect of CA in the presence of desipramine were markedly inhibited by $39.0 \pm 4.63\%$($P<0.001$, $n=11$) and $57.5 \pm 5.22\%$($P<0.001$, $n=5$) of the each control response, respectively, as shown in figure 10. Furthermore, McN-A-343 did cause a weakened reduction in CA secretion by $87.4 \pm 27.5\%$(NS, $n=6$) of the corresponding control value.

The effect of perfusion with Ca^{2+}-free medium plus EGTA on CAs release evoked by DMPP or McN-A-343

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 Kirpekar, 1975), it is of particular interest to test whether the secretory effects induced by DMPP or McN-A-343 in this presparation are also related to extracellular calcium ions. Thus, the adrenal glands were perfused with Ca^{2+}-free Krebs solution containing EGTA(5×10^{-3} M) for 30 min prior to perfusion of DMPP or McN-A-343.

Thes experimental results are shown in figure 11. Perfusion with Ca^{2+}-free Krebs solution in plus EGTA led to greatly reduction in CA release evoked by DMPP or McN-A-343 to $41.6 \pm 162.7\%$($P<0.001$, $n=8$) and $6.9 \pm 0.47\%$($P<0.001$, $n=9$) of the corresponding control responses, respectively. Ach-induced CA-secretion was also considerably decreased by $60.9 \pm 11.23\%$($P<0.001$, $n=8$) of the control response.

DISCUSSION

In isolated perfused rat adrenal preparations in which Krebs solution was infused into the perfusion stream for 60 min prior to stimulation, perfusion with DMPP or McN-A-343 for 2 min periods resulted in rapid and transient increases in the secretion of CA.

Collect and Story(1984) have found that the CA release evoked by DMPP decline abruptly between the first and second periods of DMPP exposure and this reduction may be due to agonist densensitization of the nicotinic receptors. However, in the present study, repeated perfusion of DMPP as well as McN-A-343 for 2 min three times at 30 min intervals did not produce any desensitization-like effect(tachyphyaxis) between periods. On a molar basis, McN-A-343 was about one fifth of DMPP in their relative potencies of inducing CA secretion from rat adrenal preparation. The DMPP-induced release of CA was due presumably to exocytosis of CA storage vesicles subsequent to activation of nicotinic receptors, since it was abolished by chlorisondamine and was extracellular calcium-dependent.

The secretory effect evoked by McN-A-343 was also through selective stimulation of M_1-muscarinic receptors which was blocked by atropine or pirenzepine and was calcium-dependent exocytotic mechanism.

Generally, the adrenal medulla has been employed as the model system to study numerous cellular functions involving not only noradrenergic nerve cells but also neurons. One of

such functions is neurosecretion. During the neurogenic stimulation of the adrenal medulla, Ach is released from the splanchnic nerve endings and activates cholinergic receptors on the chromaffin cell membrane(Viveros, 1975). This activation triggers a series of events known as stimulus-secretion coupling, culminating in the exocytotic release of CA and other components of the secretory vesicles in the extracellular space.

Ach, the physiological presynaptic transmitter at the adrenal medulla, releases CA and dopamine-beta-hydroxylase by calcium-dependent secretory process(Viveros *et al.*, 1986; Dixon *et al.*, 1975).

McN-A-343-evoked CA secretion was considerably inhibited by pretreatment with atropine or pirenzepine while DMPP-induced effect was rather enhanced by atropine but not by pirenzepine. Moreover, the CA secretion evoked by both secretogogues was greatly inhibited by pretreatment with chlorisondamine.

More recently, subtypes of muscarinic receptors have been recognized in many tissues(Eglen and Whiting, 1986). Receptor binding studies have supported the classification of muscarinic receptors into M_1 and M_2 on the basis of the selectivity profile of pirenzepine; receptors with a high affinity for pirenzepine are designated as M_1 and those with low affinity as M_2 receptors (Hammer *et al.*, 1980; Hammer and Giachetti, 1982). Doods and his collegues(1987) have classified muscarinic receptors into M_1(pirenzepine sensitive, neuronal), M_2(cardiac) and M_3 (smooth mucscle and glandular). In view of above studies, the present experimental results strongly demonstrate that McN-A-343 causes the secretory effect of CA through activation of M_1-muscarinic receptors in adrenal preparation.

Since chlorisondamine, a well-known autonomic ganglionic blocking agent(Gilman *et al.*, 1991), almost abolished the secretion of CA evoked by both DMPP and McN-A-343 as well as Ach, it is felt that DMPP produces the significant secretion of CA via stimulation of nicotinic cholinergic receptors in adrenal gland. However, the knotty contradictory finding that McN-A-343-evoked CA secretion was markedly reduced by chlorisondamine can be explained if chlorisondamine also blocks the muscarinic receptors in adrenal gland, as hexamethonium does. Hexamethonium has been shown to act as a nicotinic receptor antagonist and is frequently used to exclude potential nicotinic receptor interactions of muscarinic agonists such as carbachol(Barlow *et al.*, 1972). A number of workers have also suggested that hexamethonium may act directly at the muscarinic receptor in the ileum(Feddes *et al.*, 1974), ganglia(Grown *et al.*, 1980) and cardiac tissue(Rand and Staggord, 1967; Lullaman *et al.*, 1969; Leung and Mitchelson, 1982; Zonta *et al.*, 1987).

In support of this edea, the findings that physostigmine, an anticholinesterase, potentiated CA secretion induced by Ach but rather inhibited that evoked by McN-A-343 without any effects on that induced by DMPP may suggest that the secretion of CA induced by McN-A-343 is mediated through M_1-muscarinic receptors. In the present study, the fact that physostigmine blocked secretory effect evoked by McN-A-343 also supports the involvement of M_1-muscarinic activity. Moreover, It has been shown that physostigmine blocks or depresses the effect of McN-A-343 in various biological systems(Kim *et al.*, 1967; Choi, 1979; Hwang, 1967; Choi and Baik, 1981).

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 deploarizing 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.

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).

It has also been reported that cardiac glycosides increase both spontaneous and evoked CA cecretion from the perfused adrenal glands (Banks, 1967; 1970; Garcia *et al.*, 1981b; Wakade, 1981; Nakazato *et al.*, 1986) and isolated adrenal chromaffin cells(Aunis and Garcia, 1981; Sorimachi *et al.*, 1981; Pocock, 1983, a, b).

In the present study, Ach(50 ug)-evoked CA secretion may be caused through stimulation of both muscarinic and nicotinic receptors, as reported in guinea-pig adrenal gland(Nakazato *et al.*, 1988). However, DMPP-evoked CA secretion was not affected by ouabain while McN-A-343-induced effect was augmented markedly by ouabain.

It is thought that ouabain may enhance the response to muscarinic stimulation by increasing Ca^{2+} entry, which in turn increases the capacity of the intracellular Ca^{2+} pool linked to muscarinic receptors as described previously (Yamada *et al.*, 1989).

It is known that the activation of nicotinic receptors stimulates catecholamine secretion by increasing Ca^{2+} entry through receptor-linked and/or voltage-dependent Ca^{2+} channels in both perfused rat adrenal glands(Wakade & Wakade, 1983) and isolated bovine adrenal chromaffin cells(Kilpatick *et al.*, 1981; 1982; Knight & Kesteven, 1983). Thus, it is previously suggested that ouabain enhances catecholamine secretion evoked by Ach and high K^+ by increasing the rate of Ca^{2+} influx through the Ach receptor-linked Ca^{2+} channel and/or voltage-dependent Ca^+ channels on adrenal chromaffin cells as a result of the inhibition of the Na^+-K^+ pump (Nakazato *et al.*, 1986).

In view of these reports, the present study shows that enhancement of Ach-induced CA secretion by ouabain is due to an increased Ca^{2+} influx through the nicotinic receptors activation by the inhibition of the Na^+-K^+-ATPase.

It has been reported that the muscarinic re ceptor activation causes an increase in adrenal catecholamine secretion independent of extracellular Ca^{2+} in various species(Nakazato *et al.*, 1984; Wakade *et al.*, 1986; Harish *et al.*, 1987) and in cytosolic free Ca^{2+} in bovine isolated adrenal chromaffin cells without associated catecholamine secretion(Cheek & Burgoyne, 1985; Kao & Schneider, 1985; 1986; Misbahuddin *et al.*, 1985).

However, considering the present experimental results showing that McN-A-343-evoked CA secretion was enhanced by ouabain but was dependent on extracellular Ca^{2+} from the rat adrenal gland and, furthermore, DMPP-induced CA secretion was not effected by ouabain, it is felt that the present data are different from the previous report(Yamade *et al.*, 1989) and this difference may be due to different animals used in our experiment.

The indispensible role of calcium in the neurosecretory process has been well established. Yet, according to the assumptions of Baker and Knight(1978; 1980), the relationship between the concentration of intracellular calcium and the transmitter release has not been determined in the nerve terminals. As mentioned above, cacium plays the crucial role in process of depolarization-neurotransmitter release coupling in many types of secretory cells (Douglas, 1968; Schulz and Stolze, 1980; Williams, 1980).

In the present work, removal of extracellular Ca^{2+} depressed markedly CA secretion evoked by DMPP or McN-A-343 and Ach. The secretory effect of these seretogogues are apparently dependent on extracellular calcium. However, in this experiment, the reason for considerable response to DMPP and Ach in Ca^{2+}-free Krebs plus EGTA solution is not celear. McN-A-343-induced effect was clearly abolished by perfusion of calcium-free Krebs solution.

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 mere removal of extracellualr calcium. Some investigators(Boxler, 1968; Ohashi *et al.*, 1974; Casteels and Raeymeakers, 1979) reported that intracellular stores of calcium have been shown to paly some role in contraction of smooth muscle produced by noradrenaline or Ach in Ca^{2+}-free medium.

Furthermore, desipramine-pretreatment produced the marked reduction in CA secretion evoked by DMPP or Ach, but not that by McN-A-343.

It has been shown that secretions of CA evoked by Ach, excess K^+ or transmural stimulation of splanchnic nerves were reduced by desipramine or imipramine as well as by trifluoperazine(Wakade and Wakade, 1984). In general, trifluoperazine and other antidepressants inhibit the activity of calmodulin, and therefore many investigators have made use of these agents as "calmodulin inhibitors" to unravel the role of calmodulin in different

systems. Essentially in several cases, including the current study on the adrenal gland, these agents very effectively block Ca^{2+}-depedent responses. Interestingly, inhibition of a Ca^{++}-dependent response is not accompanied by a reduction in Ca^{++} uptake(Schubart *et al.*, 1980; Hequin, 1981; Douglas and Memeth, 1982; Kenigsberg *et al.*, 1982). In the present work, the findings that the secretory effect of CA evoked by Ach or DMPP but not by McN-A-343 was blocked by deipramine may support that the effects of these secretogogues are due to a calcium-dependent exocytosis. However, these was a considerable reduction in CA secretion of McN-A-343 although it was not statistically significant.

The present study shed light into characteristics and differences between DMPP and McN-A-343 in their effects on CA secretion.

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= 국문초록 =

흰쥐 적출 부신에서 DMPP 및 McN-A-343의 Catecholamine 분비작용에 관한 연구

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흰쥐 적출 부신에서 DMPP와 McN-A-343의 카테콜아민(CA) 분비작용의 차이와 특성에 대해서 연구한 결과 다음과 같다.

DMPP(100 uM)와 McN-A-343(100 uM)은 부신정맥내로 투여시 유의한 카테콜아민 분비작용을 나타내었다. Mol농도로 비교시 McN-A-343의 CA분비작용은 DMPP의 약 1/5정도였다. DMPP나 McN-A-343의 반복투여시 반응 급강현상은 관찰할 수 없었다.

DMPP의 CA분비작용은 chlorisondamine이나 desipramine또는 Ca^{2+}-free Krebs + EGTA 관류등의 전처치로 의의있게 억제되었으나, pirenzepine, ouabain 및 physostigmine등 전처치에 의해서는 영향을 받지 않았다. 그러나 atropine 전처치시 DMPP의 분비작용은 오히려 증강되었다.

McN-A-343의 CA분비작용은 atropine, pirenzepine, chloriondamine, physostigmine 및 Ca^{2+}-free medium plus EGTA 관류등의 전처치에 의해서현처히 차단되었으나 desipramine등에 의해서는 영향을 받지 않았다. 그러나 ouabain의 전치치시 McN-A-343의 분비효과는 크게 증강되었다.

이상의 실험결과로 보아 DMPP와 McN-A-343은 흰쥐 적출관류 부신에서 현저한 CA분비작용을 일으키며, 이는 Ca^{2+} 의존성 임을 보였으며, DMPP의 분비작용은 부신의 **nicotine** 수용체의 흥분을 통해서 나타내며, 또한 McN-A-343의 분비작용은 M_1-muscarine 수용체의 흥분에 의하여 유발되는 것을 생각된다. DMPP의 분비활성이 McN-A-343보다 훨씬 강력한 것으로 사료된다.