Korean J. of Pharmacology
Vol. 27, No. 2, pp. 167-181, 1991

Forskolin-Induced Potentiation of Catecholamine Secretion Evoked By Ach, DMPP, McN-A-343 and Excess K^+ From the Rat Adrenal Gland**

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ABSTRACT

The present study was an attempt to investigate the effect of forskolin on secretion of catecholamines (CA) evoked by Ach, excess K^+, DMPP, McN-A-343 and caffeine from the isolated perfused rat adrenal glands and to elucidate its mechanism of action.

The perfusion with forskolin (1.0 uM) for 1 min into the adrenal vein enhanced markedly the secreation of CA evoked by Ach (50 ug), excess K^+ (56 mM) DMPP (100 uM) and by caffeine (0.3 mM) but did not that by McN-A-343. Forskolin alone did not potentiate the CA secretion. Moreover, forskolin augmented the CA release evoked by the above same stimulation even in the absence of extracellular calcium.

The 1 min perfusion of 300 uM-dibutyryl cyclic AMP (DBcAMP), which is known to increase cyclic AMP levels, led to enhancement of Ca secretion evoked by Ach, excess K^+ and DMPP but did not that by McN-A-343 and caffeine. DBcAMP by itself also did not augment the CA secretion. In the calcium-free medium DBcAMP significantly enhanced the CA secretion by the same stimulation, except for the case of McN-A-343.

These experimental results suggest that forskolin activates adenylate cyclase, resulting the elevation of cyclic AMP which may potentiate cholinergic nicotinic receptor-mediated and also depolarization-dependent CA secretion and that it may alter the intracellular calcium homeostasis in the rat adrenal glands.

Key Words: Forskolin, Cyclic AMP, Catecholamine-Secretion, Adrenal Gland

It has been known that forskolin, a diterpene derived from the Indian plant coleus forskohlii, stimulates adenylate cyclase by a direct interaction with adenylate cylase (Seamon and Daly, 1981; 1983). The agent is one of the most powerful stimulators of the cyclase, and has thus been widely used to characterize cAMP-depen-dent mechanism (Daly, 1984). The structure of forskolin is shown as in Figure 1. Nicotinic stimulation of the adrenal medulla results in an increase of cyclic AMP levels in this tissue (Guidotti and Costa, 1974; Jaanus and Rubin, 1974; Morita *et al.* 1987; 1987). However, among the results that have been published up to date, the role of cyclic nucleotides in secretion of catecholamines (CA) is not clear, It is reported that cyclic AMP produces CA release (Peach 1972; Gutamen and Boonyaroviroj, 1979) while it has no effect on secretory function (Hohman and Perlaman, 1976; Kumakura *et al.*, 1979). Greenberg and Zinder (1982) have shown that cAMP has only a marginal stimulatory effect CA secretion.

On the other hand, forskolin, which is a potent nonhormonal activator of adenylate cycase in many tissues, is known to potentiate the stimula-

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** This study was supported by a grant from Chosun University (1991).

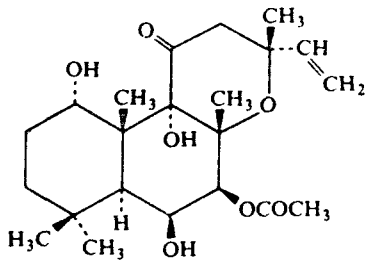

Fig. 1. The structure of forskolin.

tion-evoked CA secretion from cultured bovine chromaffin cells, suggesting that cyclic AMP inc reases stimulation-induced Ca release by enhancing calcium uptake across the cell membrane and/or altering calcium flux in an intracelluar calcium store (Morita *et al.*, 1987; 1987). Tsujimoto and his coworkers (1980) have reported that tissue cyclic nucleotides increase in the perfused dog adrenal glands before stimulation-evoked CA release. Moreover, the elevation of cyclic AMP in the perfused dog adrenal glands by various activators of adenylate cyclase is also found (Morita *et al.*, 1985). the direct addition of cyclic AMP derivatives such as 5-AMP, dibutyryl cyclic GMP, or dibutyryl cyclic AMP did not cause CA release, but enhanced CA releases evoked by nicotine, bethanechol and excess K^+, and by caffeine in the absence of extracellular calcium (Tsujimoto *et al.*, 1986).

In addition, Jeong and Choi (1989) have shown that forskolin produces a dose-related increase in the release of acetylcholine from the rabbit hippocampus by the elevation of cyclic AMP. forskolin is found to be a potent positive inotropic agent in failing human myocardium as well as in open chest dogs by activating myocardial adenylate cyclase (Bristow *et al.*, 1984). Recently, Bowling and his co-workers (1990) have reporeted that forskolin siginificantly increases cyclic AMP concentration in ventricular muscles preparations of Sprague-Dawley rats and also evelates force of contraction in muscles. However, Mchugh and McGee (1986) showed that forskolin causes an immediate, concentration-dependent inhibition of carbachol-stimulated uptake of Rb^+ through the nicotinic receptors when applied to rat pheochromocytoma (PC_{12}) cells and that these results did not appear to activation of adenylate cyclase.

It was found that the action of forskolin on Ach recepter-channel function of rat myotubes could be not associated with the cytosolic cAMP-dependent phosphorylation of Ach receptors (Grassi, Monaco and Eusebi, 1987). In addition to its action on adenylate cyclase, forskolin directly alters the gating of a single class of voltage-dependent potassium channels from a clonal pheochromocytoma cell line (Hoshi *et al.*, 1988) and it is shown that although forskolin facilitated desensitization of Ach receptors in voltage clamped rat muscle, this effect was not correlated with the abilities of forskolin and forskolin analogs to activate adenylate cyclase or phophorylate the receptors (Wagoner and Pallotta, 1988).

Hu and his investigators (1988) have shown that forskolin inhibits proteoglycan synthesis in chick embryo sternal chondrocyte cultures in a cyclic AMP-independent manner.

As aforementioned, there is clear controversy about whether various effects of forskolin are realted to the elevation of cyclic AMP concentration by activation of adenylate cyclase. Therfore, the present study was designed to investigate the effect of forskolin on stimulation of CA secretion from the isolated perfused rat adrenal gland and to elucidate its mechanism of 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 adbomen 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 to obtain enough working space for tying blood vessels and cannulations.

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 (if any), vena cava and aorta were ligated. Heparine

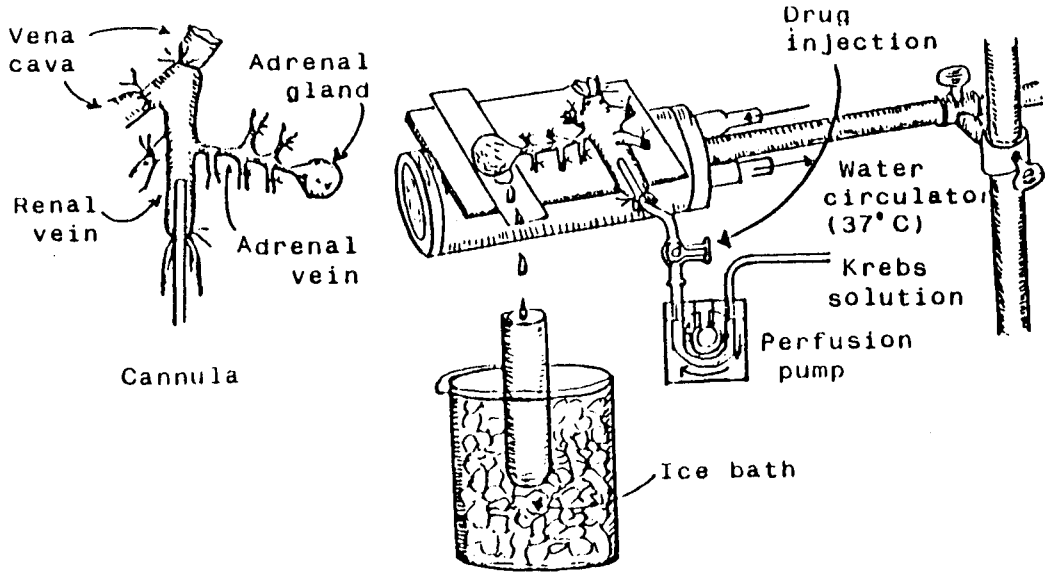

Fig. 2. Schematic drawing of the perparation used to study secretion of catecholamines in the isolated perfused adrenal gland of the rat.

(400 IU/ml) was injected into venal cava to prevent blood coagulation before ligating vessels and cannulations. A small slit was made into the adrenal cortex just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with 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 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 following composition (mM): NaCl, 118.4; KCL, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95% O_2+5% CO_2 and the final pH of the solution was maintained at 7.4±0.05. The solution contained disodium EDTA (10 ug/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of catecholamine.

Drug administration

The perfusion of DMPP (100 uM) and McN-A-343 (100 uM) for 2 minutes and and caffeine (0.3 mM) for 1 minute or single injection of Ach (50 ug) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock.

In the preliminary experiments, it was found that upon administration of the above drugs, secretory responses to Ach and KCl returned to preinjection level in about 4 min, but the responses to DMPP and McN-A-343 in 6 to 9 min. and that to caffeine lasted more than 90 min. Generally, the adrenal glands were perfused with normal Krebs solution for about one hour. The adrenal ferfusate was colected in chilled tubes.

Collection of perfusate

As a rule, prior to each stimulation with cholinergic agonists and 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 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 30 min. the amounts secreted in the "background sample" have been subtracted from those from secreted the "stimulated sample" to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effects of forskolin on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing forskolin (1 uM) for 1 min, than the perfusate was collected for a specific time period ("background sample"), and then the medium was changed to the one containing the stimulation agent and the perfusate were collected for the same period as that for the "background sample".

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using fluorospectrophotometer (Shimadzu 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 various secretogogues 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 norpinephrine (base) equivalents. All data are presented as means with their standard errors, and the significance of differences was analyzed by Student's t-test, using the computer system as previously described (Tallarida and Murray, 1987).

Drugs and their sources

The following drugs were used: forskolin, acetylcholine chloride, 1. 1-dimeth ly-4-phenyl piperazinium iodidea (DMPP), norepinephrine bitartrate (Sigma Chemical Co., U.S.A.), (3-m chlorphenyl-carbamoyloxy)-2-butynyl trimethyl ammonium chloride (McN-A-343) (RBI, U.S.A.), caffeine citrate (Mallinckrodt Chemical Words, U.S.A.) and dibutyryl cyclic AMP (Sigma chemical Co., U.S.A.). Drugs were dissovled in distilled water (stock) and added to the normal Krebs solution as required except forskolin. forskolin was dissolved in 95% ethanol and diluted appropriated (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base except the case of Ach in ug.

RESULTS

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

The basal (spontaneous) secretion of CA from the isolated perfused rat adrenal glands reached a steady stae level 60 min after the start of perfusion with nomal Krebs solution. When Ach

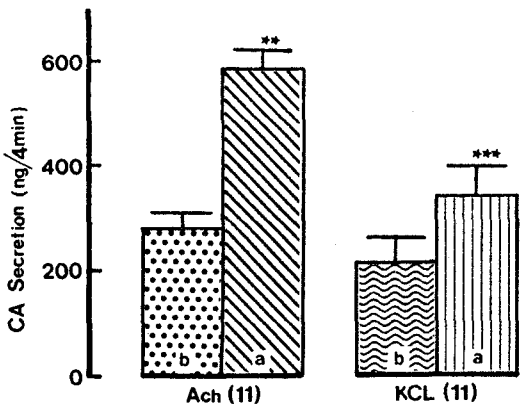

Fig. 3. Effect of forskolin on Ach and KCl-evoked release of catecholamines (CA) from the isolated perfused rat adrenal glands. CA release evoked by Ach (50 ug) and KCl (56 mM) was made after the persfusion with normal Krebs solution for one hour. "b" and "a" represent CA release evoked by Ach or KCl before (b) and after (a) the treatment with forskolin (1 uM) for 1 min. After the injection of durgs, the perfusate was cellected for 4 min. Vertical bars indicate the mean±S.E. of 11 experiments. Ach: acetylcholine. Numerals in the parenthesis denote number of experimental adrenal glands. Ordinate: the amounts of CA secretion from the adrenal gland. Abscissa: administered agents.

(50 ug) was injected into the perfusion stream via three way stopcock, amounts of CA secreted was 280.6±29.62 ng for 4 min from 5 rat adrenal glands. However, after the perfusion of forskolin, a direct activator of adenylate cyclase (Seamon and Daly. 1981; 1983; Daly, 1984) for 1min Ach evoked CA secretion was markedly enhanced to 582.6±30.96 ng ($p<0.001$), as shown in Figure 3. This result is consistent with experimental data of Morita and his colleaguses (1987; 1987). Forskolin itself did not stimulate CA secretion in the present work. From our unpublished results, it was found that the concentration of luM-forskolin produced the most powerful potentiation of CA secretion evoked by Ach as in the cultured adrenal chromaffin cells (Morita *et al.*, 1987). Therefore, in all subsequent experiments the concentration of 1.0 uM-forskolin was used with 300 uM-DBcAMP in order to compare this activiy of stimulating adenylate cyclase. CA release initiated by excess K^+ (56 mM) depolarization also was potentiated greatly by forskolin treatment. CA secretion evoked by excess K^+ before treatment wth forskolin was 216.9±44.26 ng for 4 min, but after perfusion with forskolin for 1 min was significantly enhanced to 339.4±53.88 ng ($p<0.001$, $n=11$) as shown in Figure 3.

Perfusion of adrenal glands with a selective nicotinic receptor agonist, DMPP (100 uM) for 2 min resulted in the rapid and marked increase in CA secretion. CA release evoked by DMPP (100 uM) before forskolin were 302.9±18.99 ng for the first period (0~3 min), 118.3±23.78 ng for the second period (3~6 min) and 16.9±8.25 ng for the third period (6~9 min), respectively while DMPP evoked CA secretions after the perfusion (1.0 uM) for imin were potentiated to 450.0±43.15 ng ($p<0.01$, $n=7$) for the lst (0~3 min) period and 162.9±19.37 ng, for the 2nd (3~6 min) period. Ther was no singificant increase of 20.0±4.86 ng (NS, $n=7$) during the 3rd (6~9 min) period in comparison with its control value (Fig. 4).

McN-A-343 (100 uM), which is known to be as selective Ml-muscarcarinic agonist, perfused into the adrenal gland for 2 min caused the increase in CA secretion to 65.9±4.83 ng for 4 min before forskolin, However, McN-A-343-induced CA secretion after pretretment with 1.0 uM-forskolin

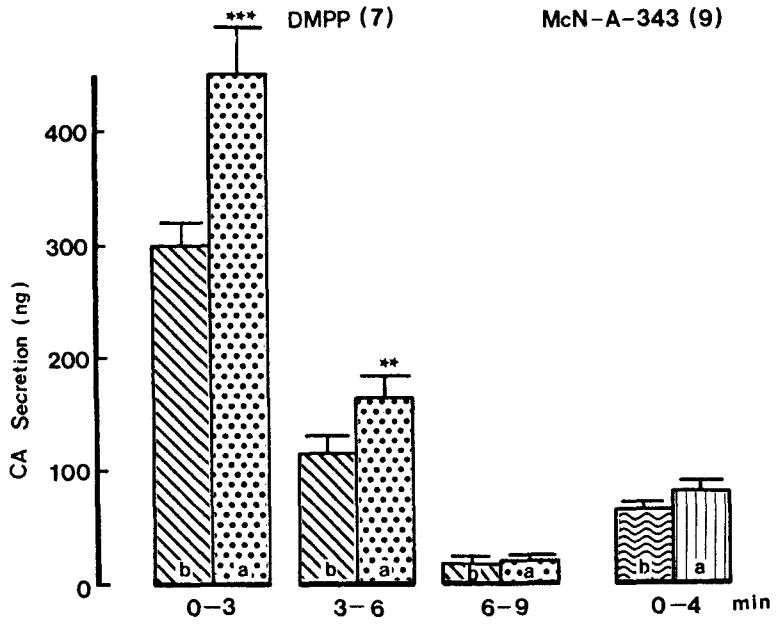

Fig. 4. Effect of forskolin on CA secretion of DMPP and McN-A-343 from the perfused rat adrenal glands. DMPP (100 ug) and McN-A-343(100 ug) were infused for 2 min in these experiments, respectively. The perfusates after administration of DMPP and McN-A-343 were collected for 9 and 4 min, respectively. Other legends and methods are the same as in Fig. 3. **: $p<0.01$, ***: $p<0.001$

for 1 min was 78.1±6.29 ng for 4 min (NS, n=9) as shown in Figure 4.

Effect of forskolin on secretion of CA evoked by caffeine (0.3 mM) from the perfused rat adrenal glands

It is known that caffeine caused a marked increase in secretion of CA fromthe isolated perfused rat adrenal glands by the mobilization of calcium from an intracellular calcium pool in the rat adrenal chromaffin cells and partly by stimulation of both muscarinic and nicotinic receptors (Lim *et al.*, 1991). It is of interest to examine the effect on forskolin of caffeine evoked CA secretion. In the present experiment, caffeine (0.3 mM) perfused into the adrenal glands produced a great and rapid increase in CA secretion which was a maximal in the first 3 min fraction of perfusate and secretory effect of CA lasted for more than 90 min as shown in Figure 5. The amounts of CA released for 30 min after 1 min perfusion of caffeine from 6 adrenal glands before perfusion with forskolin was 1986.9±145.29 (~3 min) ng, 595.8±40.81 (3~6 min) ng, 347.3± 33.50 (6~9 min) ng, 264.9±34.77 (9~12 min) ngm, 231.7±43.39 (12~15 min) ng, 177.0±44.20 (15~18 min) ng, 133.7±35.68 (18~21 min) ng, 117.6±18.51 (21~24 min)ng, 114.1±18.26 (24~27 min)ng and 96.0±18.06 (27~30 min)ng. Although caffeine induced CA secretion was clearly augmented to 2276.3±151.72 (0~3 min, $p<0.01$) ng and 685.2±48.63 (3~6 min, $p<0.05$) ng only during 6 min period after pretreatment with 1.0 uM-forskolin, CA secretion after 2nd period was rather depressed to 173.6±19.71 (6~9 min, $p<0.01$)ng and 685.2±48.63 (3~6 min, $p<0.05$) ng only during 6 min period after pretreatment with 1.0 uM-forskolin, CA secretion after 2nd period was rather depressed to 173.6±19.71 (6~9 min, $p<0.01$) ng, 150.9±16.40 (9~612 min, $p<0.01$)ng, 85.7±20.19 (12~15 min, $p<0.01$) ng, 42.5±17.36 (15~18 min, $p<0.01$)ng, 35.9±11.35 (18~21 min, $p<0.01$) ng and 18.7±7.16 (21~624 min, $p<0.01$) ng, respectively in comparison with the corresponding control value. Moreover, there is no secretion of CA during the last 6 min (24~30 min) periods.

Effect of DBcAMP on CA secretion evoked by Ach, Excess K^+, DMPP and McN-A-343 from the rat adrenal gland

It is exciting to study the effect of DBcAMP, an analog of cyclic AMP on stimulation-evoked secretion of CA secretion from the isolated per-

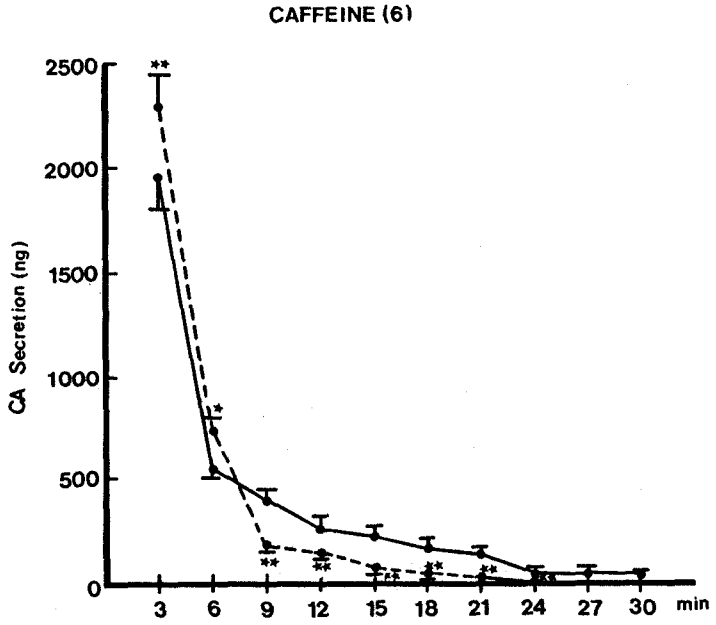

Fig. 5. Effect of forskolin on caffeine (0.3 mM)-evoked CA secretion from the perfused rat adrenal glands. Caffeine(0.3 mM) ws infused into the adrenal vein for 1 min after normal Krebs solution was perfused for 60 min. The perfused was collected for 30 min at 3 min intervals. Other legends and methods are the same as in Fig. 3. and 4. Solid line indicate the control response of caffeine before pretreatment with forskolin and dotted line the response after forskolin. * : $P<0.05$, ** : $p<0.01$

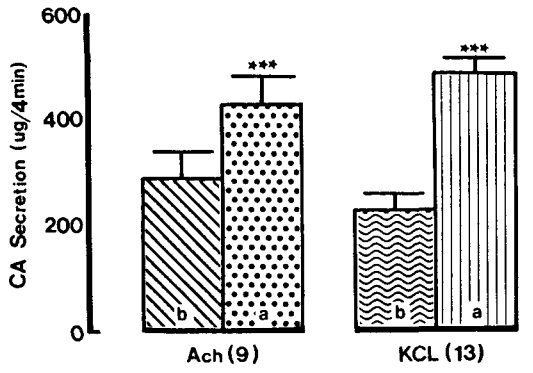

Fig. 6. Effect of dibutyryl cyclic AMP (DBcAMP) on stimulation-evoked secretion CA from the iosolated perfused rat adrenal glands. Statistical difference of CA release evoked by Ach (50 ug) or KCl (56 mM) between "before (b)" and "after (a)" the treatment with DBcAMP (300 uM) for 1 min following the perfusion of normal Krebs solution for one hour was obtained. Other legends and methods are as Fig. 3. and 4. ***: $p<0.001$

fused dog adrenal gland (Tsujimoto *et al.*, 1986). Ach (50 ug) evoked CA release before perfusion of DBcAMP was 288.8±49.25 ng for 4 min from 9 experiments but after pretreament with DBcAMP (300 uM) for 1 min was markedly enhanced to 423.6±52.31 ng/4 min ($p<0.001$), which was about 147% of the corresponding control value (Fig. 6).

When excess KCl (56 mM) in a volume of 0.05 ml was added into the adrenal gland, the CA release was 231.8±30.90 ng for 4 min, but following the perfusion with DBcAMP (300 uM) for 1 min was significantly increased to 316.4±23.25 ng/4 min ($p<0.001$) from 13 rat adrenal grands (Fig. 6). CA content secreted by perfusion with DMPP (100 uM) for 2 min was 332.0±37.79 (0~3 min) ng, 111.0±15.56 (3~6 min)ng and 18.4±7.92 (6~9 min)ng, respectively while the secretion after pretreatment with 300 uM-DBcAMP for 1 min was markedly potentiated to 490.0±42.29 (0~3 min, $p<0.001$) ng and 156.0±17.90 (3~6 min, $p<0.01$)ng without sinificant differences only at third period (6~9 min, 22.4±9.13 ng) from 10 adrenal glands (Fig. 7).

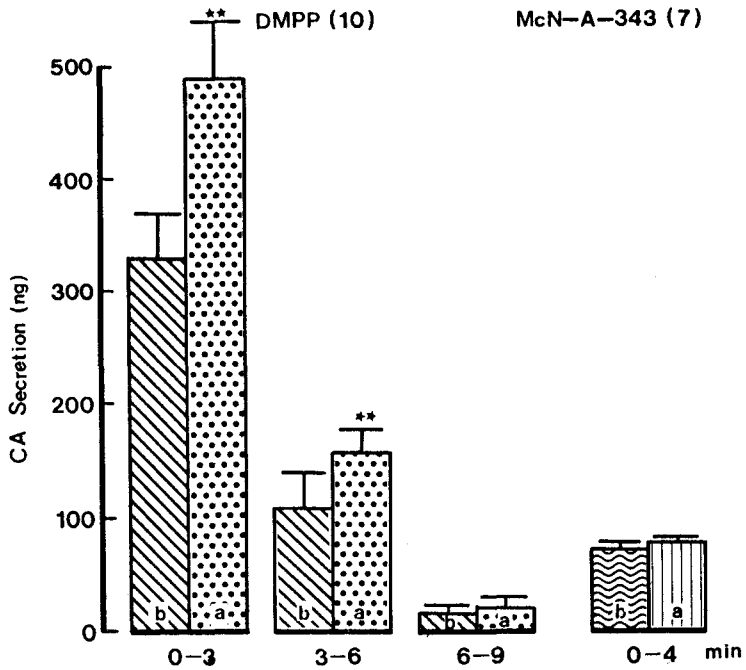

Fig. 7. Effect of DBcAMP on CA secretion evoked by DMPP and McN-A-343 from the perfusd rat adrenal glands Other legends and methods are as in Fig. 3 and 4. **: $p<0.01$

On the other hand, McN-A-343-evoked CA release was not greatly affected by DB-cAMP. CA secretion by McN-A-343 before treatment with DBcAMP was 70.4±6.96 ng/4 min but following the perfusion with DBcAMP ws 76.2±6.96 ng for 4 min from 7 experiments. There was no significant difference in McN-A-343-evoked CA between before and after DBcAMP as shown in Figure 7.

Effect of DBcAMP on caffeine-evoked CA secretion from the perfused rat adrenal glands

The effect of DBcAMP on caffeine-induced CA release was examined since caffeie is known to cause CA secretion by mobilzing calcium from intracellular calcium stores (Poisner. 1973) and DBcAMP facilitates Ca^{++} release induced by nicotine and caffeine in the absence of extacellular calclum from the isolated perfused dog adrenal gland (Tsujimoto *et al.*, 1986) and the culture bovine chromaffin cells (Morita *et al.*, 1987). In the present study, caffeine-evoked CA release for 30 min at 3 min intervals was 2114.5±172.62 (0~3 min)ng, 670.7±47.26 (3~6 min) ng, 396.4±38.77 (6~9 min) ng, 320.6±40.15 (9~12 min) ng, 254.6 ±51.59 (12~15 min) ng, 212.6±50.66 (15~18 min)ng, 164.9±44.71 (18~21 min)ng, 146.3±49.92 (21~24 min)ng, 132.0±21.85 (24~27 min) ng and 115.4±19.38 (27~30 min) ng from 5 adrenal glands while after the perfusion of DBcAMP into an adrenal vein for 1 min the secetion was 2043.6 ±186.15 (0~3 min, NS)ng, 268.3±50.46 (3=6 min, $p<0.01$)ng, 152.6±42.74 (6~9 min, $p<0.01$) ng, 96.0±19.35 (9~12 min, $p<0.001$)ng, 34.4±15. 63 (12~15 min, $p<0.001$)ng, 36.1±14.39 (15~18 min, $p<0.001$)ng, 24.8±9.15 (18~21 min, $p<0.01$) ng and 13.1±4.75 (21~24 min, $p<0.01$)ng, respectively. There was no response during 24~30 min period (Fig. 8).

Effect of forskolin of CA secretion evoked by Ach, excess K^+, DMPP, McN-A-343 in the absence of extracellular calcium

Since the physiological release of CA and dopamine bata-hydroxylase from the perfused cat adrenal gland is dependent on the extracellular calcium concnetration (Dixon, garcia and Kirpekar, 1975). It was tried to observe the in flunce of extracellular Ca^{++} on forskolin-induced potentiation to responses of secretogogues. Ach (50 ug)-and excess K^+ (56 mM)-evoked Ca seretions following the perfusion with calcium-free Krebs solution for 30 min into the gland were markedly reduced to 119.9±36.52 ($p<0.05$, n=5) and 204.8

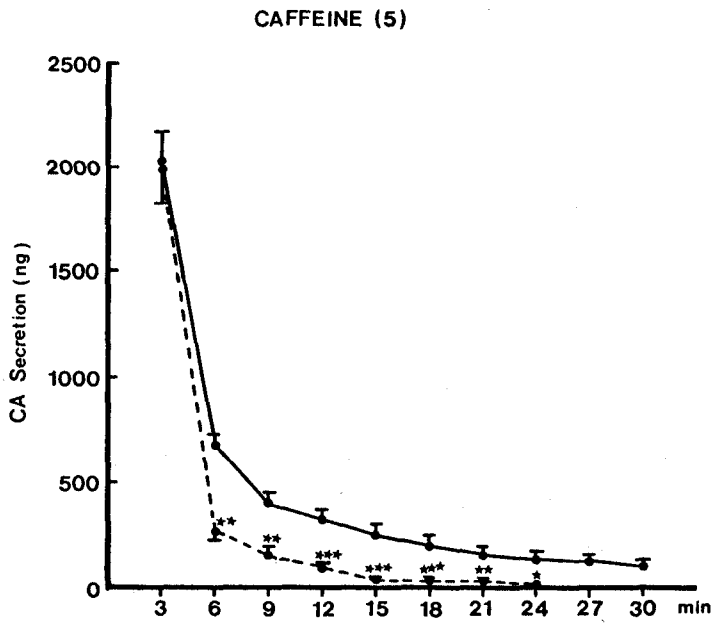

Fig. 8. Effect of DBcAMP on CA secretion evoked by caffeine(0.3 mM) from the perfused rat adrenal glands. Other legends and methods are as in Fig. 3 and 5. *: $p<0.05$, **: $p<0.01$, ***: $p<0.0001$

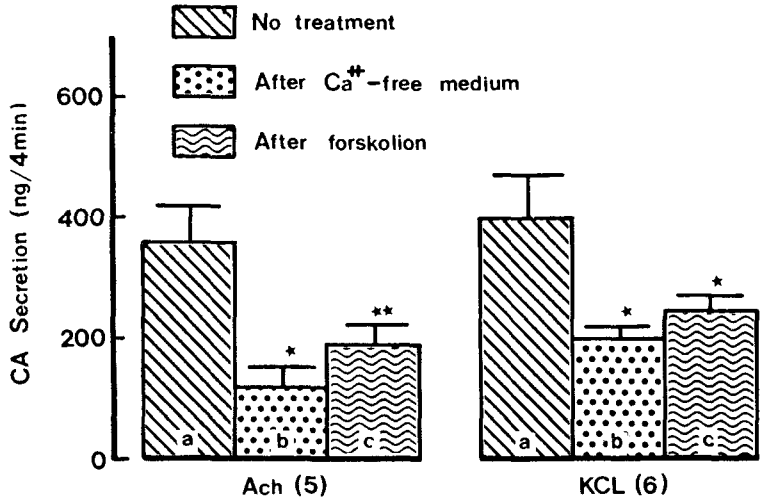

Fig. 9. Effect of forskolin on Ach-and excess K^+-evoked CA secretion in abscence of extracellular calcium from the perfused rat adrenal glands.

a: Ach-or excess K^+-evoked CA secretion without any treatment.

b: Ach-or excess K^+-evoked CA release after perfusion with Ca^{++}-free krebs solution for 30 min.

c: Ach-or excess K^+-evoked CA release after 1 min-infusion of forskolin(0.1 uM) following the perfusion with Ca^{++}-free Krebsso lution for 30 min.

Statistical difference between "a" and "b" or the between "b"and "c" was compared. Other legends and methods are as in Fig. 3 and 4. *: $p<0.05$, **: $p<0.01$

$\pm$20.43 ($p<0.05$, n=6) for 4 min respectively by comparing the corresponding control response of 358.3$\pm$60.07 ng and 397.8$\pm$68.81 ng before the treatment with Ca^{++}-free medium. However, after the perfusion with forskolin (1.0 uM) for 1 min in the absence of extracellular calcium Ach- and excess K^+-induced CA release were greatly enhanced to 183.3$\pm$36.66 ($p<0.01$, n=5) ng and 243.3$\pm$22.29)$p<0.01$, n=6) ng for 4 min, respectively in comparison with their cerressponding control values of 119.9 $\pm$ 36.52 ng and 204.8 $\pm$ 20.43 ng befor forskolin treatment (Fig. 9). DMPP (100 uM)-evoked CA secretion during 9 min period after the perfusion with calcium-free Krebs solution for 30 min was 197.5 $\pm$ 17.40 (0~3 min, $p<0.01$), 25.0$\pm$4.02 (3~6 min, $p<0.01$) and no secretion during 6~9 min, respectively as compared with the corresponding control values of 290.0$\pm$21.29 (0~3 min) ng, 82.5$\pm$17.57 (3~6 min) ng, and 11.6$\pm$4.20 (6~9 min) ng from 8 adrenal glands, respectively. However, DMPP-evoked secretion after the perfusion of forskolin for 1 min in the abscence of extracellular was clearly augmented only during 3~6 min period as shown in figure 10. Although the CA release evoked by McN-A-343 was also clearly depressed to 5.4 $\pm$ 0.92 ng ($p<0.001$) for 4 min in the absence of extracellular calcium in comparison with the control response of 70.5$\pm$6.14 ng from 6 experiments, there was no significant change in CA secretion evoked by McN-A-343 was also clearly depressed to 5.4$\pm$0.92 ng ($p<0.01$) for 4 min in the absence of extracellular calcium in comparison with the control response of 70.5$\pm$6.14 ng from 6 experiments, there was no significant change in CA secretion evoked by McN-A-343 after forskolin perfusion for 1 min in the absence of extracellular calcium as compared with the corresponding control value (Fig. 10).

Effect of DBcAMP on CA secretion evoked by Ach, exces K^+, DMPP and McN-A-343 in the absence of extracellular calcium

Since it is known that DBcAMP enhances CA release induced by caffeine in the absence of extracellular Ca^{++} from the isolated perfused dog adrenal gland (Tsujimo to *et al.*, 1986), the isolat-

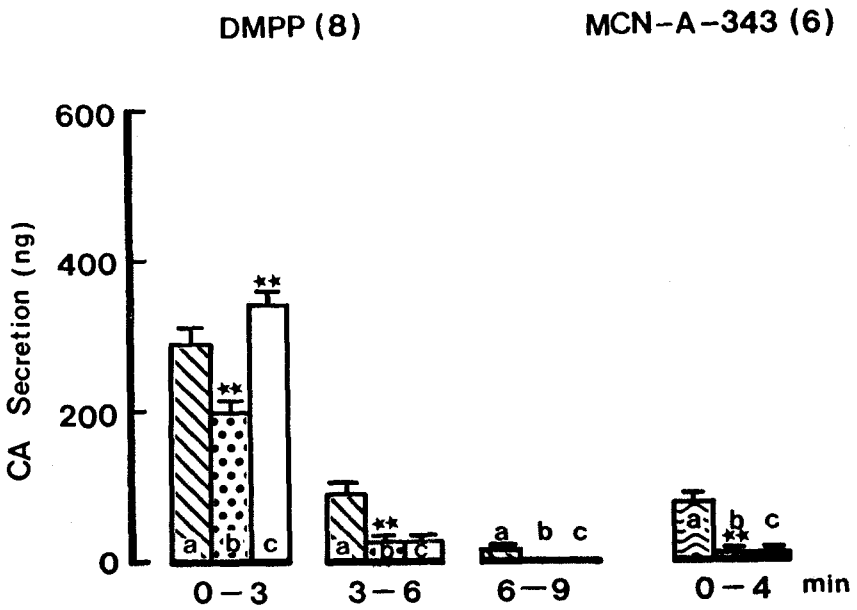

Fig. 10. Effect of forskolin on CA secretion evoked by DMPP and McN-A-343 from the perfused rat adrenal glands. Other legends and methods are as in Fig. 3, 4 and 9. **: $p<0.01$

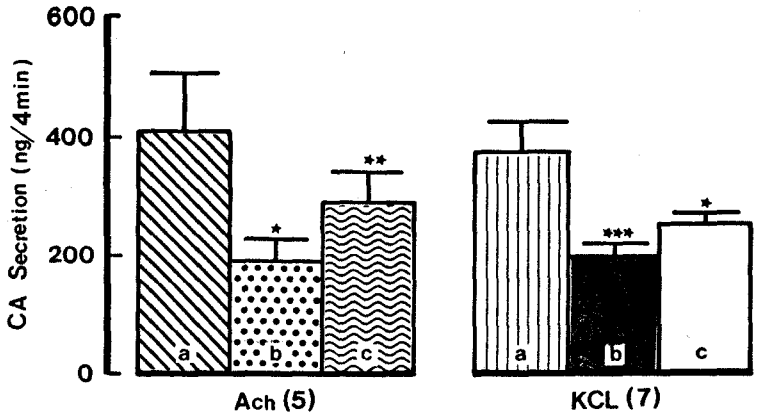

Fig. 11. Effect of DBcAMP on CA secretion evoked by Ach and excess K^+ in the absence of extracellular calcium from perfused rat adrenal glands. Other methods and legends are as in Fig. 3, 4 and 9.
C: Ach-or excess K^+-induced CA secretion after 1 min-infusion of DBcAMP(300 uM) following the perfusion with Ca^{++}-free Krebs solution for 30 min. Other legends and methods are as in Fig. 3, 4 and 9. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

ed rat adrenal gland (Lim *et al.*, 1991) and the cultured chromaffin cells (Morita *et al.*, 1991) and the cultured chromaffin cells (Morita *et al.*, 1987), it is particular interesting to examine effect of DBcAMP on stimulation-evoked CA secretion in calcium-free medium.

Ach (50 ug)-and excess K^+-evoked CA release in the absence of extracellular calcium was

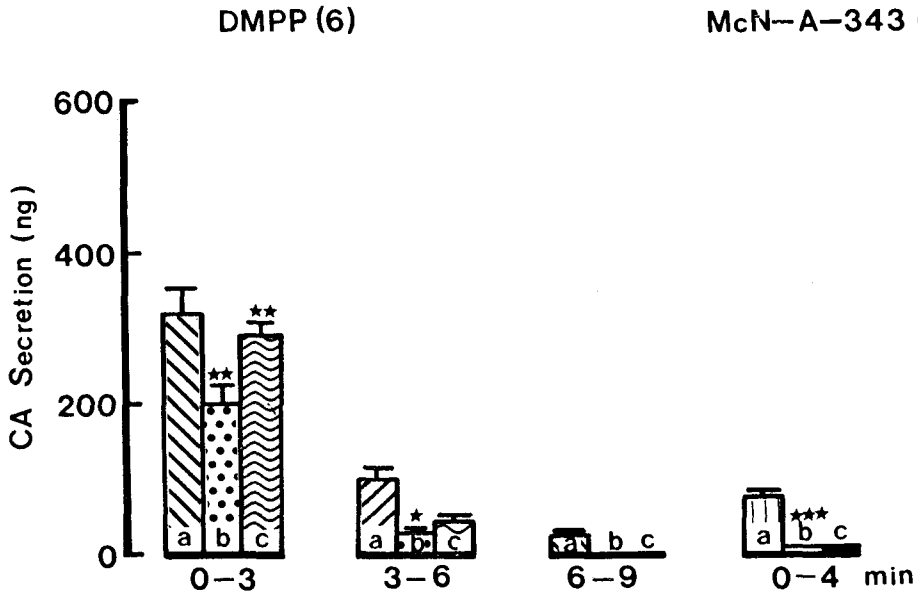

Fig. 12. Effect of DBcAMP on CA secrtion evoked by DMPP and McN-A-343 in the absence of extracellular calcium from the perfused rat adrenal glands. Other legends and methods are as in Fig. 3. 4. and 9. **<0.05, **: p<0.01, ***: p<0.001

markedly inhibited to 189.3±44.27 ($p<0.05$, n=5) ng and 197.3±18.4 ($p<0.001$, n=7) ng for 4 min, respectively as compared with each corresponding control response of 410.2±101.77 ng and 374.6±52.16 ug, while the treatment with DBcAMP (300 uM) for 1 min in calcium-free medium led to the significant enhancement in CA secretion by 286.7±51.45 ($p<0.01$, n=5) ng and 251.5±21.75 ($p<0.05$, n=7) ng, respectively (Fig. 11).

DMPP-induced Ca secretion for 9 min at 3 min intervals in Ca^{++}- free medium was clearly decreased to 202.6±24.61 (0~3 min, $p<0.01$) ng, 33.5±5.84 (3~6 min, $p<0.05$) ng and no release for last 3 min (6~9 min), respectively in comparison with their corresponding control values of 319.4±35.44 (0~3 min), 96.5±15.71 (3~6 min) and 245.9±4.62 (6~9 min). However, there was significant increase in DMPP-evoked Ca secretion of 286.3±21.43 ($p<0.01$, n=6) only for the first 3 min period after perfusion of DBcAMP for 1 min in the absence of extracellular calcium as shown in Figure 12. McN-A-343-evoked CA secretion in Ca^{++}-free medium was markedly reduced to 6.1±1.04 ($p<0.001$, n=10) ng for 4 min as compared with the control value of 74.3±6.59 ng, but there was no change in CA secretion evoked by McN-A-343 in spite of perfusion with DBcAMP (300 uM) for 1 min in the absence of extracellular calcium (Fig. 12).

DISCUSSION

In the present work, experimental data demonstrate that forskolin greatly potentiates CA secretion evoked by Ach, excess K^+, DMPP and caffeine from the isolated perfused rat adrenal glands but did not that by McN-A-343, and that they also facilitate significantly stimulation-evoked CA secretion even in the absence of extrecelilular calcium except for McN-A-343.

As aforementioned in introduction, it is shown that nicotinic receptor stimulation of the adrenal meculla causes an increase of cyclic AMP levels in tissue (Guidotti and Costa, 1974; Jaanus and Rubin, 1974). Stimulation-evoked CA release enhanced by forskolin from the perfused rat adrenal glands seems to be similar to that from the clutured bovine chromaffin cells (Morita *et*

al., 1987; 1987). In addition, the fact that DBcAMP augmented CA secretion evoked by Ach, excess, K^+, DMPP and caffeine in the present experiment is also consistent with the results of Tusjimoto *et al.* (1986). It is reported that DBcAMP increases the basal CA release in the perfused bovine adrenal gland (Peach, 1972) and in the perfused dog adrenal glands (Tsujimoto *et al.*, 1986). Michener and Peach (1984) also showed that 8-bromo cyclic AMP of a DBcAMP analog augmented slightly induced CA release from bovine adrenal gland without basal release while Jaanus and Rubin (1984) reported that DBcAMP was unable to enhance Ach-evoked CA release in the perfused cat adrenal glands. However, in the present work forskolin or DBcAMP alone did not potentiated CA secretion. It has been shown that depolarization-dependent neurosecretion from PC_{12} cells is enhanced by forskolin (Robe *et al.*, 1982), which activates the catalytic subunit of adenylate cyclase (Seamon and Daly, 1983), and that forskolin also enhances Ach-evoked Ca release from the perfused dog adrenal glands (Morita *et al.*, 1985). This finding suggests that cAMP generated immediately in chromaffin cells following Ach-receptor interaction (Tsujimoto *et al.*, 1980) may modulate CA secretion. The role of cAMP is known to be excluded in the process of CA release (Tsujimoto *et al.*, 1986). Sano *et al.* (1983) have shown that forskolin stimulates Ca^{++}-calmodulin-sensitive adenylate cyclase from rat brain. Thus in the present study it is considred that forskolin may also potentiate Ach-facilitated cAMP production by enhancing a Ca^{++}-calmodulin-stimulated adenylate cylase in adrenal chromaffin cells. Forskolin by itself induces an increase of cyclc AMP with concomittant enhancement of Ach-evoked CA release (Morita *et al.*, 1987; 1987). In the present rat adrenal glands, the finding that forskolin potentiates Ach-evoked CA release is thought to be due to an increase of cyclic AMP by activating adenylate cyclase. In support of this idea, it is known that cylcic AMP enhances Ach-evoked CA secretion in the perfused dog adrenal glands (Tsujimoto *et al.*, 1986). In contrast to this result, Wakade (1981) reported that both AMP and ATP did not produce any change in CA secretion evoked by Ach.

Anyway, the present experimental results suggest that cyclic AMP modulated stimulation-evoked CA release in adrenal chromaffin cells. Moreover, pretreatment with DBcAMP also significantly augmented Ach-evoked CA secfretion. However, the secretory effect of DBcAMP was much less potent than that of forskolin. The fact that forskolin as well as DBcAMP enhanced DMPP-evoked CA release from the perfused rat adrenal gland seems to be related to an increased cAMP from the adrenal medulla by nicotinic stimulation. It is reported that DMPP, which is a more selective agonist for autonomic ganglionic nicotinic receptors (Rang and Dale, 1987), causes a potent increase in CA secretion from rat adrenal glands through activation of nicotinic receptors (Lim *et al.*, 1991). In the present study, both forskolin and DBcAMP potentiated secretion of CA evoked not only by cholinergic nicotinic receptor agonist such as DMPP but also by excess K^+ depolarization and by caffeine, suggesting that forskolin or DBcAMP acts on some steps after interation with the cholinergic receptors including intracellular process of the release. It was well-known that the treatment of adrenal chromaffin cells with cyclic AMP analogues elevated biosynthesis of CA (Meligeni *et al.*, 1982, Haycock *et al.*, 1982) and that addition of cyclic AMP or DBcAMP to the incubation medium caused an increase in the formation of ^{14}C-DOPA in bovine adrenal medullary slices (Oka and Izumi, 1975). Furthermore, recently Houch (*et al.*, 1984) have shown that the stimulation of ^{14}C-CA synthesis induced by forskolin as well as DBcAMP is mediated through the formation of intracellular cyclic AMP and that ther is surely a direct correlation between the intracelluller cyclic AMP levels and CA bioynthesis in the isolated bovine adrenal medullary cells. In the present experiment, it is though that the elevation of cyclic AMP in chromaffin cells by forskolin or DBcAMP may result in a stimulation of CA biosynthesis that leads to an enhancement of stimulated CA secretion. In addition, Miyamoto and Oshika (1984) reported that the increase in ^{14}C-CA biosynthesis evoked by forskolin was not affected by omission of Ca^{++} from the medium. This result seems to agree with of the present work. However, this prossibility could be ruled out because forskolin facilitated the K^+-evoked release of ^{3}H-NE from the cultured bovine chromaffin cells preloaded with ^{3}H-

NE (Morita *et al.*, 1987), although forskolin or DBcAMP may possess the above dual mechanism in its secretory enhancement. Potentiation of caffeine-induced CA secretion by forskolin may be mediated hrough the stimulation of Ca^{++} mobilization from the intracellularr reservior (Poisnerr, 1973) and/or inhibition of Ca^{++} sequestration into the reservior (Rasmussen, 1982). Moreover as shown in the present study by Ach, excess K^+ and DMPP even in the absence of extracellular cvalcium could support the above results. Knadel and Schwartz (1982) have shown that cyclic AMP dependent facilitation of transmitter release in Aplysia occurs as a result of inhibition of a repolarizing K^+ conductance, which leads to prolonged depolarization and inreased Ca^{++} influx (Kandel and Schwartz, 1982; Klein *et al.*, 1982). However, enhanced release of norepinephrine in PC12 cells by forskolin was not accompanied by an increase in Ca^{++} influx (Rebe *et al.*, 1982). In the present study, interestingly it is shown that forskolin as well as DBcAMP does not enhance CA secretion evoked by a selective M_1-muscarinic agonist, McN-A-343. This finding suggests that both forskolin and DBcAMP-induced potentiaion of CA secretion evoked by Ach, excess K^+, DMPP and caffeine may be not in relation to M_1-muscarinic receptors in adrenal medulla. Anyway, it is thought that forskolin activates adenylate cyclase, resulting in the elevation of cyclic AMP which probably potentiate cholinergic nicotinic receptor mediated and also depolarization-dependent CA secretion and that it may alter the intracellular calcium homeostasis in the rat adrenal chromaffin cells.

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=국문초록=

Forkolin의 흰쥐적출관류부신으로 부터 Ach, Excess K^+, DMPP, McN-A-343에 의한 Catecholamine 분비효과의 증강작용

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Adenylate cyclase 효소를 활성화시키는 약물인 Forskolin의 흰쥐 적출관류 부신으로부터 Ach, excess K^+, McN-A-343 및 caffein에 의한 catecholamines (CA) 분비작용에 대한 영향을 검색하고, 그 기전을 규명코자 연구를 시행하여 다음과 같은 연구결과를 얻었다.

Forskolin (1.0 uM)은 흰쥐 부신적출정맥내로 1분동안 관류시킨 후 Ach(50 ug), excess K^+(56 mM), DMPP (100 uM) 및 caffeine (0.3 mM)에 의한 CA 분비작용을 현저히 증강시켰으나 McN-A-343에 의한 CA분비작용에는 영향을 미치지 않았다. Forskolin 자체는 CA분비작용을 일으키지 못하였다. 또한 세포의 calcium을 제거한 상태에서도 위 약물에 의한 CA분비작용에 대하여 유의한 증강작용을 나타내었다. 그러나 McN-A-343의 CA작용에는 영향이 없었으나 위의 약물의 CA분비작용을 유의하게 강화시켰다.

Cyclic AMP를 증가시키는 약물로 알려져 있는 dibutyryl cyclic AMP (DBcAMP)는 300 uM 농도를 1분간 관류시 Ach, excess K^+ 및 DMPP의 CA 분비작용을 뚜렷하게 증강시켰으나 McN-A-343 및 caffeine의 CA분비에는 별다른 영향이 없었다. DBcAMP 자체도 CA분비작용에는 영향을 미치지 못하였으나 또한 DBcAMP는 세포의 칼슘제거시에도 위의 약물에 의한 CA분비작용을 의의있게 증강시켰다. 그렇지만, McN-A-343의 CA분비작용은 증강시키지 못하였다.

이상의 연구결과로 보아 Forskolin는 adenylate cyclase를 활성화 시킴으로써 cyclic AMP 농도를 증가시켜 세포내로 칼슘유입을 증강시키며, 또한 세포내의 칼슘이동에도 관여함으로써 cholinergic nicotinic stimulation 및 depolarization에 의한 CA분비작용을 상승시키는 것으로 사료되어진다.