Studies on Secretion of Catecholamines evoked by Panaxadiol in the Isolated Rabbit Adrenal Gland

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ABSTRACT

The effect of Panaxadiol(PD), which is an active component of Korean Ginseng Saponins, on the secretion of catecholamines (CA) from the rabbit adrenal gland and its mode of action were investigated in the present study. PD (400μg) increased significantly the secretion of CA from the isolated perfused rabbit adrenal gland. PD-induced secretion of CA was reduced markedly by treatment of atropine, CA secretion induced by Ach or PD was potentiated significantly by physostigmine-treatment. Chlorisondamine did inhibit CA secretion of PD or Ach. Perfusion of PD (400μg) for 30 min enhanced the secretory activity of CA by Ach. Ouabain weakened the secretory response induced by PD but rather enhanced the response by Ach. Adenosine-treatment resulted in marked enhancement of CA secretion by PD or Ach, Pefusion with Ca^{2+}-free Krebs containing EGTA (5 mM) for about 30 min totally blocked secretory effect induced by Ach and also weakened that by PD. From the above experimental results, it is suggested that PD causes secretion of catecholamines from the rabbit adrenal gland by a calcium-dependent exocytotic mechanism. The secretory effect of PD is due to the stimulation of cholinergic muscarinic and nicotinic receptors present in the adrenal gland and partly to a direct action on the chromaffin cell itself.

Key Words: Panaxadiol, Adrenal gland, Catecholamine-secretion.

INTRODUCTION

The root and rhizoma of several species of Panax are used as drugs in chinese medicine. Korean Ginseng, the root of Panax ginseng C.A. Meyer, is best-known and widely used among the people of East and South East Asian Countries since ancient times as a healthful tonic, stimulant or aphrodisiac (Hu, 1976; Fulder, 1976; Veninga, 1973). In general, acid hydrolysis of Ginseng Saponin mixture resulted in formation of two main sapogenins named Panaxadiol and Panaxatriol along with oleanolic acid as a minor sapogenin (Shibata, 1974).

It is known that Ginseng contains consituents which are able to exert many entirely different pharmacological actions. Despite of the fact that much work particularly in recent years has been performed to elucidate the pharmacological action of Ginseng many problems still remain to be solved.

Thus it has been suggested that more pharmacological studies with individual, chemically well-defined constituents from Ginseng are particularly necessary. Various pharmacological effects of Ginseng are known at present.

In a series of experiments of pharmacological actions of Ginseng, especially on blood pressure, the effort has been made to clarify the mechanism of pressor or depressor response of Ginseng.

It is clear that effects of Ginseng on blood pressure are very controversial. As far as the action of Ginseng extract on blood pressure is concerned, some investigators (Lee et al., 1976; Lee, 1974; Hsu, 1956; Lee and Cho, 1971; Oh et al., 1968; Ozaki et al., 1963) showed hypotensive action

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while Kitagawa and Iwaki(1963) found hypertensive responses. Wood et al. (1964), Park(1960) and Petkov(1961) described that Ginseng extract caused a biphasic action on blood pressure, namely transient fall followed by prolonged elvation. Recently, Lim et al. (1987) also found that total Ginseng saponin produced the pressor and the depressor actions in the rat, and that its depressor response is exerted partly through the stmulation of cholinergic muscarinic receptors with the blockade of adrenergic alpha-receptors, and that the pressor response is caused by stimulation of nicotinic receptors in autonomic ganglia. Some investigators (Sohn et al., 1979; 1980; Seok et al., 1981) reported that Ginseng, when given in small amounts in spontaneously hypertensive rat (SHR), caused pressor action but relatively large dose of it revealed rather dose-dependent depressor action with decreased plasma renin activity. Siegel (1979) described that various commercial Ginseng preparations including roots, capsules, tablets, teas, extracts, cigarettes, chewing gum and candies, when administered to some American people regularly for two years caused hypertensive action and central nervous system stimulation, and concluded that long-term ingestion of large amounts of Ginseng should be avoided. Sokabe et al. (1984) reported that administration of Korean Red Ginseng powder for 11 weeks had no effect on blood pressure in normotensive Donryu(DON), spontaneoulsy hypertensive and renal hypertensive rats, wherase it slightly increased blood pressure in deoxycorticosterone salt hypertensive rats. Lim et al. (1987) also reported that total Ginseng saponin increases secretion of catecholamines (CA) in the isolated perfused rabbit adrenal gland via direct action on chromaffin cells which was partly mediated by muscarinic receptors.

On the mechanism of those hypotensive action, there are many reports, including action by serotonin antagonism, by Ca^{++} -antagonism, by histamine releases, and the direct action, etc. However, not much evidence is available to clarify the hypertensive mechanism. Thus, there are many disagreements among the pharmacological effects of Ginseng extract on the blood pressure depending on authors or constituents.

Therefore, in order to clarify the principal mechanism of effects of Ginseng on the blood pressure, especially pressor action, it seems to be very interesting to reexamine the effects of Panaxadiol(PD), one of chemically well-defined contituents of Ginseng, on the secretion of CA from adrenal medulla, which causes catecholamine secretion by nerve stimulation or cholinomimetics, and regulates circulatory CA to maintain cardiovascular homeostasis and sodium balance in normal and hypertensive individuals through the renin-angiotensin system.

MATERIALS AND METHODS

Experimental animals

White mature male rabbits, weighing 1.7-2.8 kg, were used in this experiment. Animals were tied in supine position on the fixing pannel to prevent their movements without anesthetization. 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 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 to the entrance of the adrenal vein. Perfusion of the gland was started, making 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 $37°C \pm 1°C$(B). The right adreneal gland was also removed in the same way as in the case of the left adrenal gland.

Perfusion of the adrenal gland

The adrenal glands were perfused by means of a lsco pump at a rate of about 0.8 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;
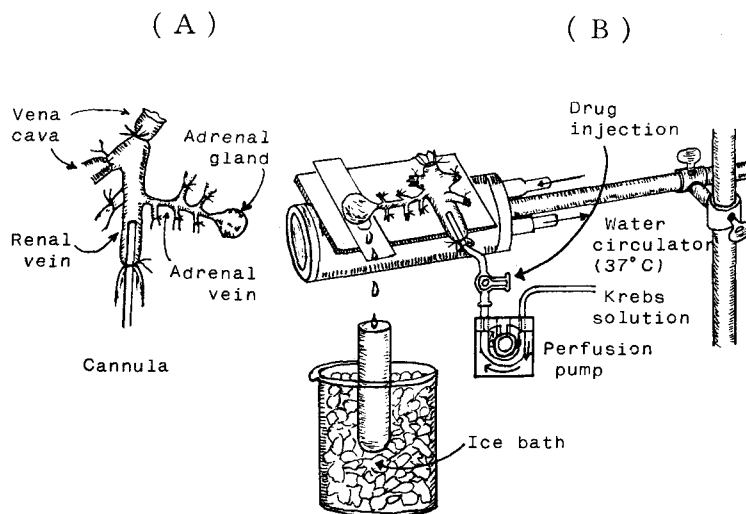
(A) (B)

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

NaHCO$_3$, 25; KH$_2$PO$_4$, 1.2; glucose, 11.7. The soultion was bubbled with 95% O$_2$+5% CO$_2$ and the final pH was 7.4±0.5. The solution contained disodium EDTA (10μg/ml) and ascorbic acid (100μg/ml) to prevent oxidation of catecholamine.

Panaxadiol (PD) and acetylcholine (Ach) injection

400μg of PD or 50μg of Ach 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 forementioned doses of PD or Ach, the secretory response returned to preinjection level in about 4 min. after administration of PD or Ach. Generally, the adrenal gland was perfused with Krebs solution at least for more than an hour before stimulation. The adrenal perfusate was collected in chilled tubes. Details of the collection of samples are given in the Results section.

Analysis of CA

CA content of perfusate was measured, directly by the fluorometric method of Anton and Sayre(1962), without the intermediate purification on alumina, using Fluorospectrophotometer (Shimazu Co.). A volume fo 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by Ach or PD 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-stimulate 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

Drugs used in this experiment were acetylcholine chloride, adenosine, norepinephrine bitartrate, physostigmine sulfate, ouabain, EGTA (Sigma Chemical Co.), and atropine sulfate (Merk), chlorisondamine chloride (CIBA, Co.). PD was prepared from Korean Panax Ginseng by modified Shibata's method.

RESULTS

CAs secretion in response to Ach or PD of the adrenal gland

Secretion of CA evoked by PD or Ach at the given dosage is shown in Fig. 2. About one hour after perfusion of the adrenal gland with Krebs solution the injection of 400μg-PD or 50μg-Ach into the perfusion stream caused significant secre-
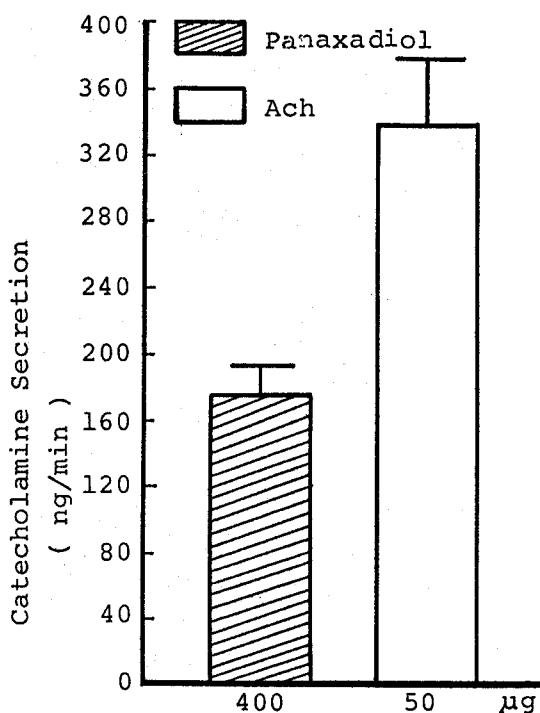
Fig. 2. Secretion of catecholamine in response to Panaxadiol or acetylcholine from the adrenal gland. 30 min after the beginning of perfusion with Krebs solution, Panaxadiol (400 μg) and acetylcholine (50 μg) were injected at 30 min intervals into the perfusion stream, respectively. The averaged values from 39 and 30 experimental trials, respectively, were obtained. After injection, the perfusate was for 4 min. Vertical bars represent S. E. of mean. Ordinate ; the amounts of catecholamine secreted from the adrenal gland. Abscissa : doses of Panaxadiol and acetylcholine. Ach ; acetylcholine.

Fig. 3. Inhibition percentages of catecholamine secreted by Panaxadiol and acetylcholine after atrpinization. Secretion of catecholamin was evoked 30 min after perfusion of the adrenal gland with krebs solution containing 2.3 μM-atropine. The results are obtained after compring each value with its corresponding control group. Numeral in bracket indicates number of animals tried in this experiment. Other legend are the same as in fig. 2. ** ; p < 0.01.

tion of great amounts of CA over the background secretion. The net CA secretion during 4 min in the the case of 400μg-PD was 174.07±17.7 ng/min (P<0.001), and in the case of injection with 50μg-Ach, the release was 339.31±38.2 ng/min of CA(P<0.001). From our unpublished results, it was found that dose of 400μg-PD produced most significant secretory response of CA among 100, 200, 400, and 800μg of PD used. Therefore, in all subsequent experiments a dose of 400μg-PD was used with a dose of 50μg-Ach in order to compare their results. It was found that these results were similar to those of the study which used total Ginseng sapoin (Lim *et al.*, 1987), although dosa-
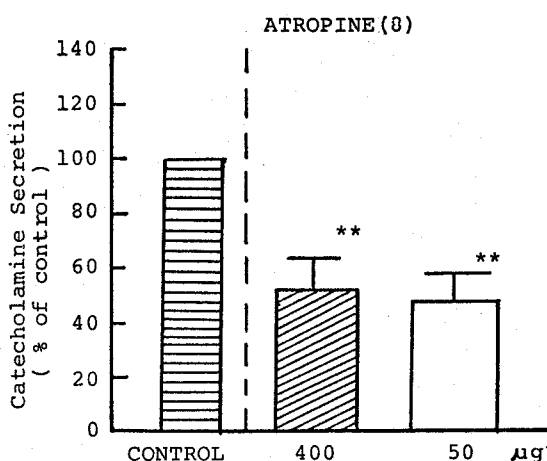
ges used were different.

Effect of atropine on the release of CAs induced by PD

PD could be releasing Ach from the presynaptic sites in the adrenal medulla in part through the stimulation of muscarinic receptors. Therefore, the secretory effect of PD was tested in gland pretreated with 2.3μM-atropine, an antimuscarinic agent. Fig. 2 shows that the PD-and Ach-evoked CA releases were clearly reduced by atropine-treatment. In 8 rabbits, responses to 200μg-PD and 50μg-Ach in the presence of atropine were 48.43± 15.4%(P<0.01) and 52.35±9.8(P<0.01) of the estimated control responses(100%), respectively.

Effects of physostigmine on the release of CAs induced by PD

Since PD-induced CA secretion was reduced by atropine. PD may be releasing Ach form presynaptic sites in the adrenal medulla, and Ach released might be rapidly degraded by acetylcholinesterase. Therefore, it is of interest to observe the interrelationship between PD and physostigmine(Eilman *et al.*, 1985), which is an acetylcholinesterase inhibitor. When PD and Ach are
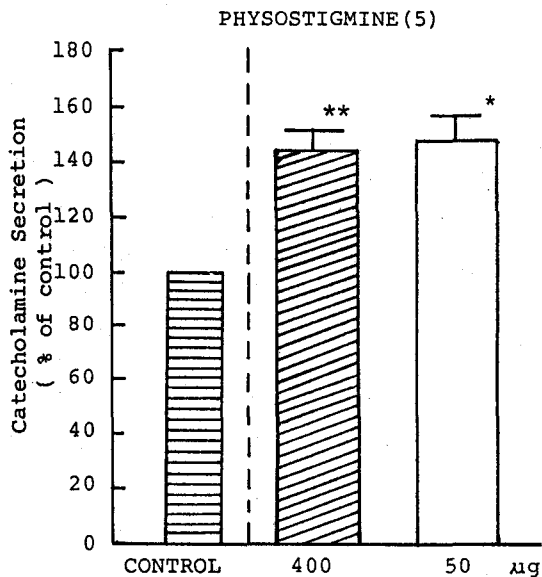
PHYSOSTIGMINE(5)

Fig. 4. Effect of physostigmine on secretion of cate-
cholamine evoked by Panaxadiol and acetylcho-
line in the isolated perfused rabbit adrenal
gland. 30 min after perfusion of krebs solution
containing 0.01 μM-physostigmine, the perfusa-
te to Panaxadiol or acetylcholine was collected
for 4 min. Other legends are the same as in fig.
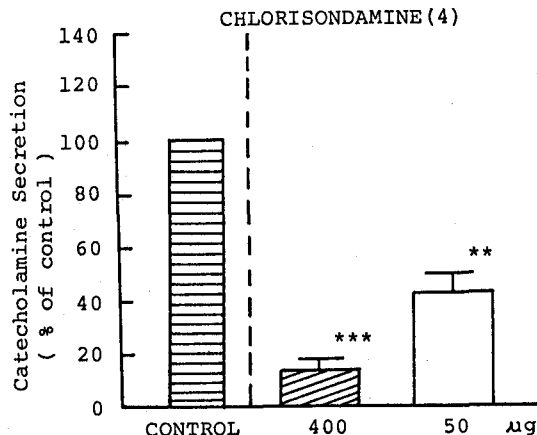2 and 3. * ; p < 0.05, **, ; p < 0.02.

CHLORISONDAMINE(4)

Fig. 5. Effect of chlorisondamine on catecholamine
evoked by Panaxadiol and acetylcholine. Se-
cretion of catecholamine was induced 30 min
after perfusion of krebs solution containing
1.0 μM-chlorisondamine. Other legends are
the same as in fig. 2 and 3. ** ; p < 0.01, ***;
p < 0.001.

introduced 30 min after the start of 0.01 μM-phso-
stigmine treatment, CA output evoked with Ach
was markedly increased to $148.73 \pm 8.6\%$(P<0.02)
of non-treated response, but that evoked by PD
was also potentiated to $145.50 \pm 11.7\%$(P<0.05) of
the corresponding control.(Fig. 4)

Effects of chlorisondamine on the release of CAs induced by PD

In the light of the fact that PD-evoked CA
release was blocked by treatment of atropine and
potentiated by physostigmine, the CA secretion
induced by PD could be secondary to the release of
Ach produced by the drug from the presynaptic
cholinergic nerve terminals present in the adrenal
medulla. That this is not the case if shown by the
experiments depicted in Fig 5. In each of 4 experi-
ments, both glands from the same aniaml were
perfused with Krebs solution in the presence of the
ganglionic blocking agent, 1.0μM-chlorisonda-
mine (Gilman et al., 1985). CA secretion evoked
by PD and Ach were markedly inhibited by 87.
$33 \pm 6.7\%$(P<00.1) and $56.38 \pm 4.5\%$ (P<0.01),

respectively, of the each corresponding control.

Effect of ouabain on CA secretion induced by PD or Ach

It has been demonstrated that cardiac gly-
cosides induce 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, 1973b) and the per-
fused cat adrenal gland(Garcia et al., 1980). Since
ouabain blocks Na$^+$-pump and is well known to
produce similar effects in several test system, it was
decided to investigate the effects of ouabain on CA
secretion evoked by PD or Ach in the rabbit
adrenal gland. In 10 experiments, after obtaining
control secretion, the adrenal gland was perfused
with 2.0μM-ouabain for 30 min and the secretory
response was evoked in the presence of ouabain.
As shown in Fig. 6, secretory response evoked by
PD was depressed significanly by $82.48 \pm 6.1\%$
(P<0.001) of the control, while CA release in-
duced by Ach was markedly potentiated by 155.
$14 \pm 45.11\%$(P<0.01) of the control.

Effect of adenosine on CA secretion evoked by PD or Ach

Since the data obtained in Fig. 3, 4 and 5.
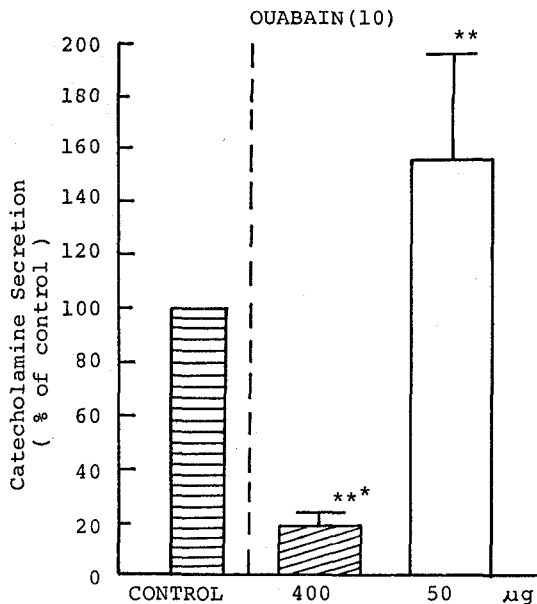indicated that CA secretion evoked by PD was

OUABAIN (10)

Fig. 6. Effect of ouabain on catecholamine-secretion by Panaxadiol and acetylcholine. Catecholamine-secretion was induced 30 min following perfusion of krebs solution containing 2.0 μM-ouabain into the adrenal gland. Other legends are the same as in fig. 2. and 3. ** ; p < 0.01, *** ; < 0.001.
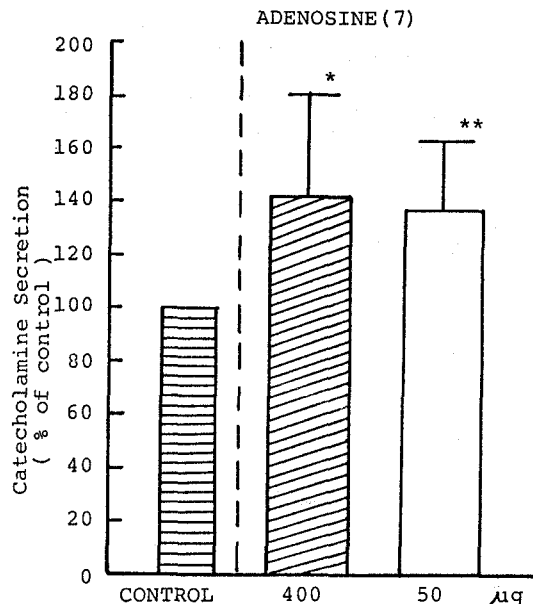
ADENOSINE (7)

Fig. 7. Effect of adenosine on secretion of catecholamine evoked by Panaxadiol and acetylcholine in the isolated perfused rabbit adrenal gland. Catecholamine-secretion was produced following perfusion of krebs solution containing 0.18 mM-adenosine for 30 min. Other legends are the same as in fig. 2. and 3. * ; p < 0.05, ** ; p < 0.01.

induced partly by the action of Ach released through excitation of muscarinic and nicotinic receptors on chromaffine cells, it was therefore of particular interest to study the effect of adenosine on CA release evoked by PD or exogenous Ach. The results of these experiments are shown Fig. 7. In each of 6 experiments, perfusion of the adrenal gland with 18 mM-adenosine for 30 min resulted in significant increases in CA secretion evoked by Ach or PD which were $149.0 \pm 25.3\%$ (P < 0.01) and $142.03 \pm 43.7\%$ (P < 0.05) of the each corresponding control, respectively.

Effect of prolonged perfusion with Ca⁺⁺-free medium plus EGTA on CA secretion evoked by PD

According to Dixon et al. (1975), since the physiological release of CA and dopamine beta-hydroxylase from the perfused cat adrenal gland is dependent on the extracellular calcium concentration, it was of interest to examine whether the secretory effect induced by PD in this preparation of the rabbit was also related to extracellular
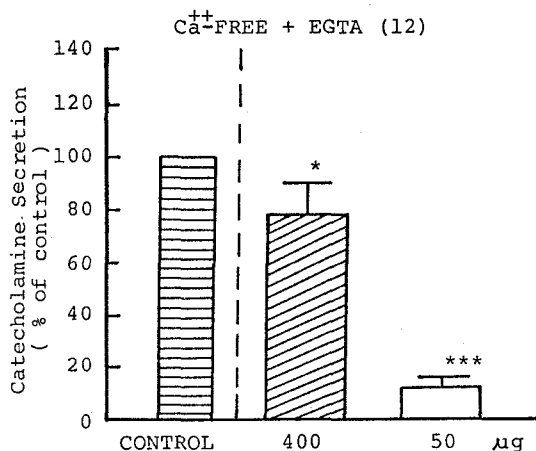
Ca^{++}FREE + EGTA (12)

Fig. 8. Effect of perfusion with calcium-free krebs solution plus EGTA on catecholamine-secretion evoked by Panaxadiol or acetylcholine. Secretion of catecholamine was evoked following perfusion of calcium-free krebs solution containing 5.0 mM-EGTA for 30 min. Other legends are the same as in fig. 2 and 3. * ; p < 0.05, *** ; p < 0.001.
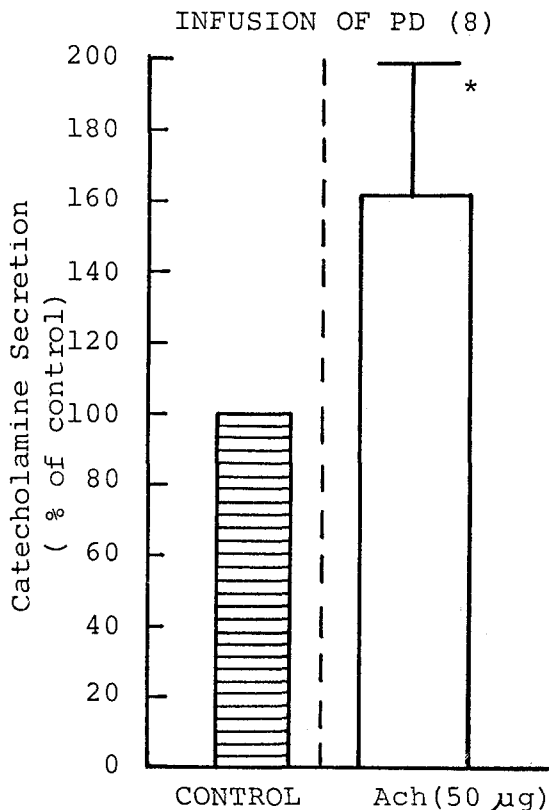
INFUSION OF PD (8)

Fig. 9. Effect of infusion of Panaxadiol on acetylcholine -induced secretion of catecholamine from the rabbit adrenal gland. Secretion of catecholamine was evoked 30 min after perfusion of krebs solution containing Panaxadiol (400 μg/ 30 min) into the gland. Other legends are the same as in fig. 2. and 3. * ; p < 0.05.

calcium ions. Therefore, adrenal glands were perfued with Ca^{2+}-free Krebs solution containing 5 mM-EGTA for 30 min. Fig. 8 shows the influence of Ca^{2+}-free medium plus EGTA on secretory responses evoked by PD or Ach. In 12 experiments, perfusion of gland with this solution for 30 min led to almost complete disappearance of the response evoked by Ach at $88.4\pm4.8\%(P<0.001)$ of control, while CA secreted by PD was depressed merely by about 25% of the control.

Effect of infusion of PD on Ach-induced secretion of CAs

As forementioned in Fig. 3 and 4, it was thoguht that PD may exert CA secretion by muscarine-like action in adrenal gland. Therefore, it seems to

be interesting to test whether PD will be able to enhance Ach-activitiy in secretion of CA. Ach-evoked secretion of CA after perfusion of Krebs solution containing PD(400μg/30 min) for 30 min. is shown in Fig. 9. In 8 experiments, CA secretion induced by Ach in the presence of PD was enhanced significantly by $169.4\pm41.2\%$ of the control $(P<0.05)$.

DISCUSSION

The present experiments clearly demonstrate that PD produces release of CA from the isolated perfused rabbit adrenal glands, and that this secretory response evoked by PD is critically dependent on the extracellular calcium concentration, and that this effect of PD was related to the stimulation of nicotinic and muscarinic receptors present in the chromaffin cell of the adrenal gland. These findings also show that mechanism of CA secretory action of PD is little different from that of total Ginseng saponin as shown in the previous work.(Lim et al., 1987).

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 neurogenic stimulation of the adrenal medulla, acetylcholine (Ach) is released from splanchnic nerve endings and activates 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 catecholamines 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 finding that PD exerts release of CA from the adrenal gland suggests that this effect of PD may be related to induction of hypertension following chronic administration of Ginseng preparation as shown in previous reports(Kitagawa and Iwaki, 1963; Wood et al., 1964; Sohn et al., 1979; Sohn et al., 1980; Seok et al., 1981; Siegel, 1979). Now, the site of action of PD as a CA secretory agent in the adrenal medulla will be discussed. Ach, the physiological

presynatic transmitter at the adrenal medulla, releases CA and dopamine beta-hydroxylase by a calcium dependent secretory process(Dixon et al., 1975; Viveros et al., 1968). Since PD also has cholinergic action in some other biological systems(Takagi, 1974), a question arises whether secretion of CA evoked by PD in the rabbit adrenal gland is secondary to the release of Ach from cholinergic nerve terminals present in the gland.

In the present study, chlorisondamine(Gilman et al., 1985), a well-known ganglionic blocking agent did significantly interfere with the secretory response to PD. It is thought that perfusion of PD may cause the relase of Ach by depolarizing splanchnic nerve terminals, which in turn activates nicotinic(and probably muscarinic) receptors present in the chromaffin cell to evoke CA secretion.

However, pretreatment with physostigmine (Gilman et al., 1985), a reversible anticholinesterase, before administration of PD markedly potentiated the secretory effect of PD as well as Ach.

Moreover, pretreatment of atropine also inhibited significantly secretory effect of PD. Perfusion of PD also caused markedly the potentiation of CA secretion evoked by Ach.

These observations strongly suggest that PD may produce the release of CA through the activation of muscarinic receptors with nictoinic 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.

In the present experiment, the fact that chlorisondamine attenuated the secretory effect of PD indicates that the action of PD may be exerted via nicotinic Ach receptors located on the surface of the chromaffin cell with activation of muscarinic receptors. Garcia and his colleagues(1980) reported that ouabain-induced release of CA was enhanced by physostigmine perfusion. This result represented that the influence of eserine on CA secretion evoked by PD is very similar to that of

ouabain. Moreover, it was also found that ouabain perfusion weakened markedly secretory effect of PD in this experiment.

Adenosine perfusion made significant enhancement of CA secretion evoked by both exogenous Ach and PD. Generally, it is known that adenosine inhibits norepinephrine release from sympathetic neurons as well as acetylchoine 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 on 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 with these reports, adenosine potentiated markedly CA secretion induced by Ach or PD in present experiment. However, this effect of Ach by adenosine perfusion has been already reported in our previous study(Lim and Choi, 1986), in which adenosine enhanced the action of Ach on CA secretion via adenosine receptors on chromaffin cells. And it seemed that there is a species difference in the modulation of adnosine for CA secretion from the adrenal gland.

Since the secretory effect of CA by PD was potentiated by adenosine perfusion as in the case of Ach, it is thought that effect of PD may also be related to adenosine receptors.

The indispensible role of calcium in the neurosecretory process has been throughly established. According to the assumptions of Baker et al.(1978; 1980), the relationship between concentration of intracellular calcium and transmitter release has not yet been determined in nerve terminals; but, interestingly enough, 10^{-6} is level of intracellular calcium that gives half-maximal release of catecholamines from leaky bovine adrenal medullary cells. As mentioned above, calcium plays the crucial role in many secretory mechanisms. Furthermore, there appears to be important parallels between depolarization-neurotransmitter release coupling at nerve terminals and depolarization-secretion coupling in many other types of secretory cells(Douglas, 1968; Schulz and Stolze, 1980; Williams, 1980). In the present experiment removal of extracellular calcium depressed CA secretion evoked by either PD or Ach. However,

although the secretory response evoked by Ach was almost extinguished in Ca²⁺-free Krebs solution, that evoked by PD was maintained at the level of about 75% of the control in zero Ca²⁺ medium. In the case of cat adrenal, Douglas and Rubin(1963) observed over 99% blockade of C A out flow in response to ACh in Ca²⁺-free Lock's solution. In perfused bovine adrenal glnd(Phillipu and Schumann, 1962) and bovine adrenal slices (Oka et al., 1965), a complete blockade of CA secretion by Ach in Ca²⁺-free medium as observed in the present experiment was seen. Ishikawa and Kanno(1978) observed over 95% inhibition of adrenaline secretion from perfused rat adrenal gland in response to Ach in the absence of calcium.

In the present study, the reason for the considerable response to PD in Ca²⁺-free Krebs soultion are not clear. It may be that chromaffin cells of the rabbit adrenal gland contain an intracellular store of calcium which participates in the secretion of CA as shown in the rat adrenal gland(Baker and Knight, 1978). Such a store may not be easily depleted by removal of extracellualr calcium. Some investigators(Bozler, 1967; Ohshi 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 Ca²⁺-free medium. Since PD 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 PD is achieved by a rise in the intracellular ionized calcium concentration. In the present work, the secretory effect of CA evoked by PD was depressed markedly by perfusion of oubain(Schwarta, 1976; Aker, 1977), which is a well-known specific inhibitor of sodium, potassium-activated ATPase in many biological systems. This findings show possibility that PD could cause CA release through the mode of action to be similar with that of ouabain. Garcia et al. (1980) showed that ouabain release CA from the perfused cat adrenal gland by a calcium-dependent exocytotic mechanism, which is due to a direct action of 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.

If this is the case, it may be that inhibition of the sodium pump by PD as in the case of ouabain will ultimately lead to intracellular sodium ac-

cumulation and potassium loss, and that such movovalent cation redistribution may cause a rise of intracellular ionized calcium level first, by depolarization of chromaffin cells or seretion, and by activation of the sodium-dependent calcium influx system. Moreover it is found that Ginseng inhibits Na⁺-K⁺ ATPase in some biological systems (Kim et al., 1977; Chough et al., 1979; Kim et al., 1980; Lee et al., 1985).

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 neruons and chromaffin cells induces a sharp secretory response which rapidly desensitizes of adrenergic neurons and chromaffin cells induces a sharp secretory response which rapidly desensitizes, 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 PD was a short-acting one (data and 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.

REFERENCES

Akera T: Membrane adenosin triphosphatase; a digitialis receptor?, Science, NY, 198:567-574, 1977

Anton AH and Sayre DA: A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138:360, 1962

Baker PF and Knight DE: Caclium-dependent exocytosis in bovine adrenal meduallary cells with leaky plasma membrane. Nature 276:620-622, 1978

Baker PF and Knight DE: The relation between ionized calcium and cortical granule exocytosis in eggs of the sea urchin Echinus esculentus. Pro R Soc London Ser 207:149-161, 1980

Baker PF and Rink TJ: Catecholamine release from bovine adrenal medulla in response to maintained depolarization. J Physiol 253:593-620, 1975

Banks P: The effect of ouabain on the secretion of catecholmine and on the intracellular concentration of potassium. J Physiol 193:631-637, 1967

Bozler E: Role of calcium in initiation of activity of smooth muscle. Am J Physiol 216:671-674, 1967

Brandt BL, Hagiwara S, Kidokoro Y dnd Miyazaki S:

Action potentials in the rat chromaffin cell and effects of acetylcholine. *J Physiol* 263:417-439, 1976

Casteels R and Raeymaekers L: *The action of acetylcholine and catecholamines on an intracellular calcium store in the smooth muscle cells of the guinea pig taenia coli. J Physiol* 294:51-68, 1979

Chough YS, Kim ND and Kwon YW: *A study on the effect of ginseng saponin on rat intestinal mucosal Na+, K+-ATPase. Yakhak Hoeji* 22:120-127, 1979

Dixon WR, Garcia AG and Kirpekar SM: *Release of catecholamines and dopamine beta-hydroxylase from the adrenal gland of the cat. J Physiol* 244:805-824, 1975

Douglas WW: *Stimulus-secretion coupling: The concept and clues from chromaffin and other cells. Br J Pharmacol Chemother* 30:612-619, 1968

Douglas WW, Kanno T and Sampson SR: *Effects of acetylcholine and other medullary secretogogues and antagonists on the membrane potential of adrenal chromaffin cells:an analysis employing techniques of tissue culture. J Physiol* 188:107-120, 1967

Douglas WW and Poisner AM: *On the mode of action of acetylcholine in evoking adrenal medullary secretion: increased uptake of calcium during the secretory response. J Physiol* 162:385-392, 1962

Douglas WW and Rubin RP: *The mechanism of catecholamine release from the adrenal medulla and the role of calcium in stimulus-secretion coupling J Physiol* 167:288-310, 1963

Fredholm BB and Hedqvist P: *Modulation of neurotransmission by purine nucleotide and nucleosides. Biochim. Pharmacol* 29:1635-1643, 1980

Fulder S: *About Ginseng, London, Thorsons Publishers Ltd,* 1976

Garcia AG, Hernandez M, Horga JF and Sanchez-Garcia P: *On the release of cetecholamine and dopamine betahydroxylase evoked by ouabain in the perfused cat adrenal gland. Br J Pharmacol* 68:571-583, 1980

Garcia AG and Kirpekar SM: *Release of noradrenaline from slice of cat spleen by pretreatment with calcium, stropantium and barium. J Physiol* 235:693, 1973b

Garcia AG, Kirpekar SM and Sanchez-Garcia P: *Release of noradrenaline from the cat spleen by nerve stimulation and potassium. J Physiol* 261:301-317, 1976

Gilman AG, Goodman LS and Gilman A: *The Pharmacological Basis of Therapeutics,* 7th ed, New York, 1985, p110, p215

Hsu ST: *Panax Ginseng Var. noto-ginseng. Jap J Pharmacol* 6L18-28, 1956

Hu SY: *The genus Panax(ginseng) in Chinese medicine. Economic Botany* 30:11-28, 1976

Ishikawa K and Kanno T: *Influence of extracellular calcium and potassium concentration on adrenal release and membrane potential in the perfused adrenal medulla of the rat. Jap J Physiol* 28:275-289, 1978

Kim HJ, Lee JW and Kang DH: *Effect of Panax Ginseng alcohol extract on activity of Na+, K+-activated ATPase Ca2+ activated ATPase in several biological membranes. Yonsei J Med Sci* 20(1):116-124, 1977

Kim ND, Kim CK, Kim BK, Han BH and Lee SS: *A study on the tonic effects of ginseng saponins on the rat heart. Yakhak Hoeji* 24(1):15025, 1980

Kitagawa H and Iwaki R: *Pharmacological study on Panax Ginseng. Fol Pharmacol Jap* 59(5):348-345, 1963

Lee KS: *The pharmacological actions of Ginseng on Cardiovascular system, Proceedings of the 1st international Ginseng symposium, p57, Korea Ginseng Research Institute, Seoul, Korea,* 1974

Lee SB and Cho KC: *Influence of Ginseng on the experimental renal hypertension. J Catho Med Coll* 20:89, 1971

Lee SW, Lee JS and Jin KD: *Effects of ginseng saponin on the activity, phosphorylation, 3H ouabain binding of purified Na, K-ATPase isolated from the outer medulla of sheep kidney Yakhak Hoeji* 29(2):76-89, 1985

Lee WC, Chang WS and Lee SK: *Histamine-liberating action of Ginseng. New Med J* 3:37, 1976

Lim DY and Choi CH: *Potentiation of acetylcholine-induced secretion of catecholamines by adenosine from the adrenal gland. Med J Chosun Univ* 11(1):83-95, 1986

Lim DY, Park KB, Kim KH, Man JK, Lee KS, Kim YK, Chung YH and Hong SP: *Influence of Total Ginseng saponin on the blood pressure of the rat. SOONHWANKI* 17(3):409-417, 1987

Lim DY, Park KB, Kim KM, Lee KS, Man Man JK and Kim YH: *Influence of total Ginseng saponin on secretion of catecholamines in the siolated adrenal gland of rabbits. Korean Biochem J* 20(3):230-238, 1987

Lindmar R and Loffelholz K: *The neuronal efflux of noradrenaline; dependency on sodium and faciliation by ouabain. Naunyn-Schmiedebergs Arch Pharmacol* 284:93-100, 1974

Oh JS, Lim JK, Park CW and Han MJ: *The effect of*

Ginseng on experimental hypertension. Korean J Pharamacol 4:27, 1968

Oh a shi H, Takewaki T and Okada T: Calcium and the contractile effect of carbachol in the depolarized guinea pig taenia caecum. Jap J Pharmacol 24:601-611, 1974

Oka M, Ohuchi T, Yoshida H and Imaizumi R: The importance of calcium in the release of catecholamine from the adrenal medulla. Jap J Pharmacol 15:348-358, 1965

Ozaki J, Nakajima T and Takamori T: Hypotensive constituents of Panax Ginseng. Fol Pharmacol J ap. 59:27, 1963

Ozawa H and Katsuragi T: Ouabain-induced potentiation on the contractions of guinea-pig vas deferens. Eur J Pharmacol 25:147-154, 1974

Park DI: Pressor and depressor actions of Panax Ginseng in mammals. Korea Med J 5:18, 1960

Petkov W: Pharmacological studies of the drug Panax Ginseng. Arznainmittel Forschung 11:418, 1961

Phillipu A and Schummann HJ: Der Einfluss von Calcium auf die Brenzcate chinamin freisetzung. Experientia 18:133-140, 1962

Phills JW and Wu PH: Roles of adenosine and its nucleotides in the central synapic transmission. Prog Neurobiol 16:187-239, 1981

Schulz I and Stolze HH: The exocrine pancrease; The role of secretogogues cyclic nucleotides and calcium in enzyme secretion. Ann Rev Physiol 42:127-156, 1980

Schwartz A: Is the cell membrane Na+, K+-ATPase enzyme system the pharamcological receptor for digitalis?. Circulation Res 39:2-7, 1976

Seok SE, Park CH, Nam SH, Choi HS, Lee JI, Lee DH, Huh BY and Soh ES: An experimental study on the antihypertensive effects of Korea Ginseng to spontanously hypertensive rats(SHR) in labile stage of hypertension. J Korean Med Assoc 24(6):509-515, 1981

Shibata S: Some chemical studies on Ginseng. proceedings of the international Ginseng symposium, p69-76, The central Rearch Institute, office of Monopoly, Korea, 1974

Siegel RK: Ginseng abuse syndrome. JAMA 241:1614-1615, 1979

Sohn ES, Park SC, Huh BY, Lee CK, Rhim HK, Ham JS, Yang CM, Ham CS, Park CW and Kim HJ: An animal experiental study on the effect of Ginseng on blood pressure and plasma renin activity in spontaneously hypertensive rat. J Korean Med Assic 22(9):731-745, 1979

Sohn ES, Park SC, Huk BY, Lee DH, Rhim HK, Young CM, Han CS, Song BS, kim SJ, Park CW and Kim HJ: An animal experiemental study of the effect of Korean Ginseng on body weight and blood pressure in spontaneously hypertensive rat with oral administration. J Korea Med Assoc 23(1):37-48, 1980

Sokabe H, Kishi K and Watanabe TX: Effects of Korean Red Ginseng powder(GP) administered orally on blood pressure in hypertensive rats, Proceeding of the 4th international ginseng symposium, p57. Korean Ginsing and Tobacco Ressearch Institute, Seoul, Korea, 1984

Takagi K: Pharmacological studies on ginseng. Proceedings of international ginseng sympsoim, The central research institute, Office of Monopoly, Republic of Korea, Seoul, p119-127, 1974

Veninga L: The Ginseng Book, Feltin, Calif, Big Tree Press 1973

Viveros OH: Mechanism of secretion of catecholaminies from adrenal medulla. In Handbook of physiology, Endocrinology. Vol VI, Sect 7. The adrenal gland. American physiological society, Washington DC, pp389-426, 1975

Viveros OH, Arqueros LC and Kirshner N: Release of catecholamines and dopamine beta-hydroxylase from the adrenal medulla. Life Sci 7:609-618, 1968

Wakade AR and Wakade TD: Inhibition of noradreanaline release by adenosine. J Physiol 282:45-49, 1979

Wakade AR: Studies on secretion of catecholamines evoked by acetylcholine or transmural stimulation of the rat adrenal gland. J Physiol 313:463-480, 1981

Williams JA: Regulation of pancreatic acinar cell function by intracellular calcium. Science 177:1104-1105, 1980

Wood WB, Roh BL and White RP: Cardiovascular actions of Panax Ginseng in dogs. Jap J Pharmacol 14(3):284-294, 1964

== 국문초록 ==

파낙사디올의 가토적출부신의 카테콜아민 분비작용에 관한 연구

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본 연구에서 한국산 인삼의 활성성분의 하나인 Panaxadiol(PD)에 대한 가토적출부신에서 카테콜아민(CA)의 분비작용과 작용기전을 파악하고자 실험을 시행하여 다음과 같은 결과를 얻었다.

PD(400μg)을 가토적출부신에 투여하였을 때 카테콜아민의 분비를 의의있게 증가시켰다. PD의 이러한 CA 분비작용은 atropine 처치로 현저히 억제되었다.

Physostigmine 전처치시 PD 뿐만 아니라 Ach의 CA 분비작용은 뚜렷이 증가되었다. 그러나 chlorisondamine 전처치로 PD나 Ach의 분비효과는 억제되었다. 또한 PD(400 μg/30 min)을 주입한 후에 Ach의 CA 분비 효과는 오히려 강화되었다. PD나 Ach의 작용은 adenosine 전처리시 현저히 증강되었다. EGTA(5 mM)와 함께 Ca-free Krebs액으로 30분 주입한 경우에 Ach의 분비작용은 거의 전적으로 차단되었으며, PD의 작용도 약화되었다.

이상의 실험결과로 보아, PD는 가토적출부신에서 Ca^{++} 의존적으로 CA 분비를 증가시키며, 이러한 작용은 cholinergic muscarinic 및 nicotinic receptor의 흥분작용에 기인하며, chromaffin cell에 대한 일부 직접작용도 개재되어 나타나는 것으로 사료된다.