

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

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### Abstract

The effect of panaxadiol (PD), 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. PD (400  $\mu$ g) increased significantly the secretion of CA from the isolated perfused rabbit adrenal gland. PD-induced secretion of CA was reduced markedly by treatment with atropine. CA secretion induced by Ach or PD was potentiated by physostigmine treatment. Chlorisondamine inhibited CA secretion of PD or Ach. Perfusion of PD (400  $\mu$ g) for 30 minutes enhanced the secretory activity of CA by Ach. Ouabain weakened the secretory response caused

by PD but enhanced the response by Ach. Adenosine treatment resulted in marked increase in CA secretion by PD or Ach. Perfusion with calcium free Krebs solution containing 5 mM EDTA for 30 minutes completely blocked the secretory effect induced by Ach and also weakened that evoked by PD. It is suggested that PD causes the secretion of CA from the rabbit adrenal gland by a calcium dependent exocytotic process. The secretory effect of PD is due to the stimulation of cholinergic muscarinic and nicotinic receptors present in the adrenal gland partly by direct action on the chromaffin cell.

### 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 constituents 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 actions 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 elevation. 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 stimulation 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 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), spontaneously hypertensive and renal hypertensive rats, whereas 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 release, 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 re-examine the effects of Panaxadiol (PD), one of chemically well-defined constituents of ginseng, on the secretion of CA from adrenal medulla, which cause 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.

## Material and Method

**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-soaled gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and for 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 the branches of the adrenal vein, the renal vein (if any), vena cava and aorta were ligated. Heparine (400IU/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  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (B). The right adrenal 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 lscop 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;  $\text{CaCl}_2$ , 1.18;  $\text{NaHCO}_3$ , 25;  $\text{KH}_2\text{PO}_4$ , 1.2; glucose, 11.7. The solution was bubbled with 95%  $\text{O}_2$  + 5%  $\text{CO}_2$  and the final pH was  $7.4 \pm 0.5$ . The solution contained disodium EDTA (10 $\mu\text{g}$ /ml) and ascorbic acid (100 $\mu\text{g}$ /ml), to prevent oxidation of catecholamine.

**Panaxadiol (PD) and acetylcholine (Ach) injection:** 400 $\mu\text{g}$  of PD or 50 $\mu\text{g}$  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 preinlec-

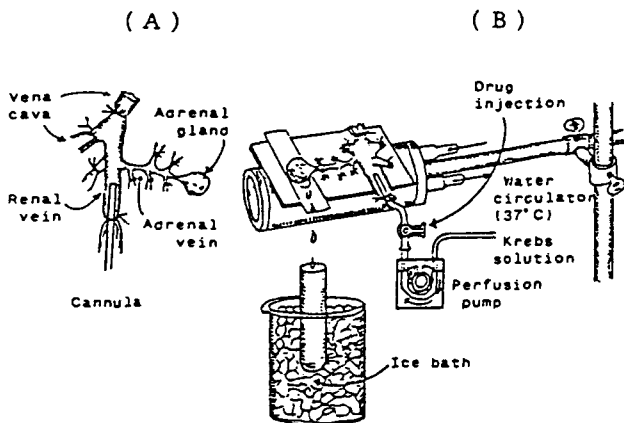


Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamine in the isolated perfused adrenal gland of the rabbit.

tion level in about 4 min. Therefore, each sample was collected for 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 for samples are given in the Results section.

**Analysis of CA:** CA content of perfusate was measured, directly by the fluorimetric method of Anton and Sayre (1962), without the intermediate purification on alumina, using Fluorospectrophotometer (Shimazu Co.). A volume of 0.2 ml of the perfusate was used for the reaction.

The CA content in the perfusate of stimulated glands by Ach or 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-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents. All data are presented as means with their standard errors, and differences were compared by student's paired "t" test.

**Drugs:** 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

**CA 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 400 $\mu\text{g}$ -PD or 50 $\mu\text{g}$ -Ach into the perfusion stream caused significant secretion of great amounts of

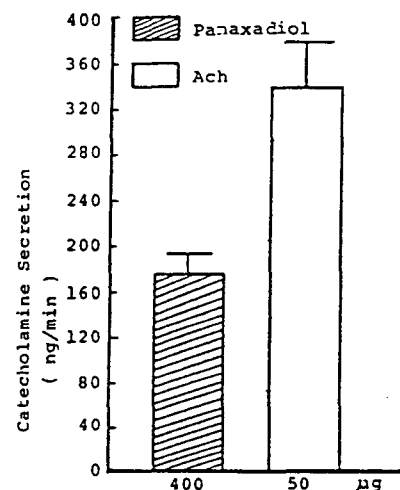


Fig. 2. Secretion of catecholamine in response to Panaxadiol or acetylcholine from the adrenal gland. 30 min after the beginning of perfusion with Krebs solution, Panaxadiol (400  $\mu\text{g}$ ) and acetylcholine (50 $\mu\text{g}$ ) were injected at 30 min intervals into the perfusion stream, respectively. 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.

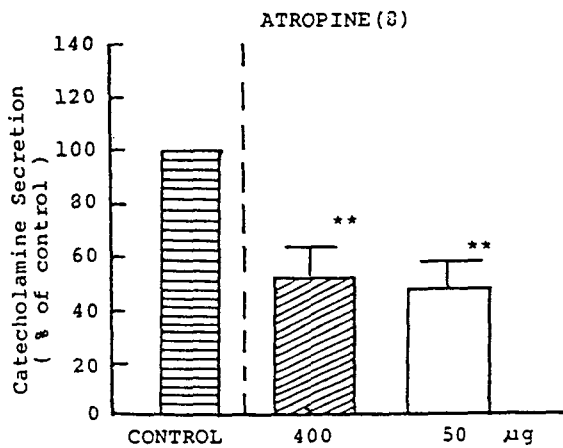


Fig. 3. Inhibition percentages of catecholamine secreted by Panaxadiol and acetylcholine after atropinization. Secretion of catecholamine was evoked 30 min after perfusion of the adrenal gland with Krebs solution containing 2.3  $\mu$ M-atropine. The results are obtained after comparing each value with its corresponding control group. Numeral in bracket indicates number of animals tried in this experiment. Other legends are the same as in fig.2 \*\*:P<0.01.

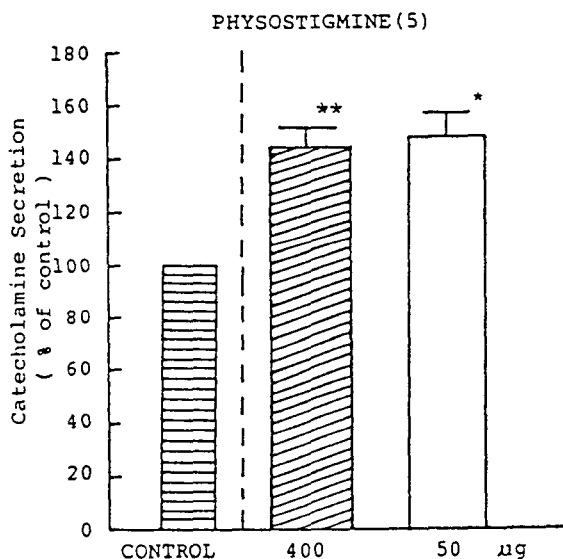


Fig. 4. Effect of physostigmine on secretion of catecholamine evoked by Panaxadiol and acetylcholine in the isolated perfused rabbit adrenal gland. 30 min after perfusion of Krebs solution containing 0.01  $\mu$ M-physostigmine, the perfusate to Panaxadiol or acetylcholine was collected for 4 min. Other legends are the same as in fig. 2 and 3. \*: P<0.05. \*\*:P<0.02.

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

**Effect of atropine on the release of CA 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 2.3  $\mu$ M-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 200 $\mu$ g-PD and 50 $\mu$ g-Ach in the presence of atropine were 48.43 $\pm$ 15.4% (P<0.01) and 52.35 $\pm$ 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 from 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 (Gilman et al., 1985), which is an acetylcholinesterase inhibitor. When PD and Ach are introduced 30 min after the start of 0.01 $\mu$ M-physostigmine 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 is shown by the experiments depicted in Fig. 5. In each of 4 experiments, both glands from the same animal were perfused with Krebs solution in the presence of the ganglionic blocking agent, 1.0 $\mu$ M-chlorisondamine (Gilman et al., 1985). In this case CA secretion evoked by PD and Ach were markedly inhibited by 87.33 $\pm$ 6.7% (P<0.001) and 56.38 $\pm$ 4.5% (P<0.01), respectively, of the each corresponding control.

**Effects of ouabain CA secretion induced by PD or Ach:** It has been demonstrated that cardiac glycosides induce the release of CA in the perfused bovine adrenal

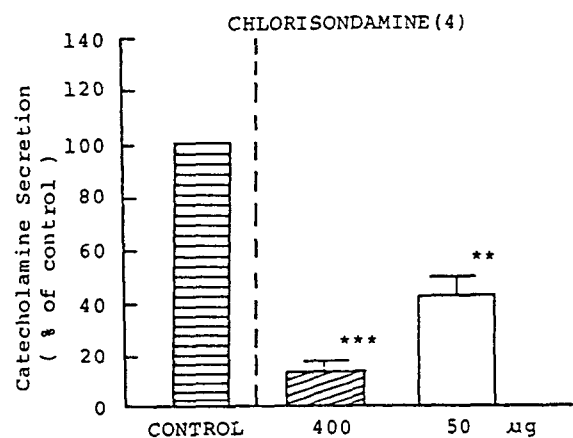


Fig. 5. Effect of chlorisondamine on catecholamine evoked by Panaxadiol and acetylcholine. Secretion of catecholamine was induced 30 min after perfusion of Krebs solution containing 1.0  $\mu$ M-chlorisondamine. Other legends are the same as in fig. 2 and 3. \*\*:P<0.01. \*\*\*:P<0.001.

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 perfused cat adrenal gland (Garcia et al., 1980).

Since ouabain blocks  $\text{Na}^+$ -pump and is well known to produce similar effects in several test systems, 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,

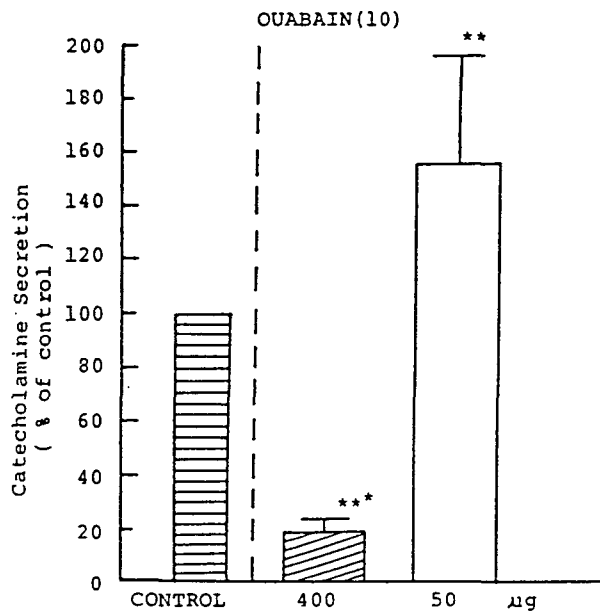


Fig. 6. Effect of ouabain on catecholamine-secretion by Panaxadiol and acetylcholine. Catecholamine-secretion was induced 30 min following perfusion of Krebs solution containing  $2.0 \mu\text{M}$ -ouabain into the adrenal gland. Other legends are the same as in fig. 2 and 3. \*\*:  $P < 0.01$ . \*\*\*:  $P < 0.001$ .

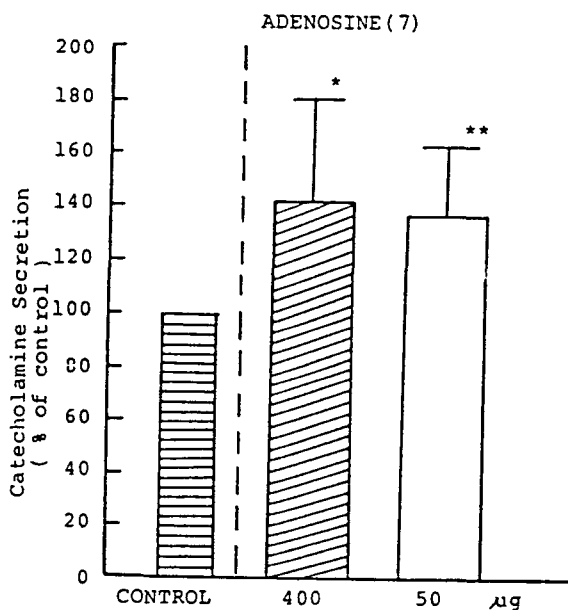


Fig. 7. Effect of adenosine on secretion of catecholamine evoked by Panaxadiol and acetylcholine in the isolated perfused rabbit adrenal gland. Catecholamine-secretion was produced following perfusion of Krebs solution containing  $0.18 \text{ mM}$ -adenosine for 30 min. Other legends are the same as in fig. 2 and 3. \*:  $P < 0.05$ . \*\*:  $P < 0.01$ .

the adrenal gland was perfused with  $2.0 \mu\text{M}$ -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 significantly by  $82.48 \pm 6.1\%$  ( $P < 0.001$ ) of the control, while CA release induced 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 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 in Fig.7. In each of 6 experiments, perfusion of the adrenal gland with  $18 \text{ mM}$ -adenosine for 30 min resulted in the 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  $\text{Ca}^{++}$ -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 calcium ions. Therefore, adrenal glands were perfused with  $\text{Ca}^{++}$ -free Krebs solution containing  $5 \text{ mM}$ -EGTA for 30 min. Fig.8 shows the influence of  $\text{Ca}^{++}$ -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  $88.4 \pm 4.8\%$  ( $P < 0.001$ ) 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 thought that PD may exert CA secretion by muscarin-like action

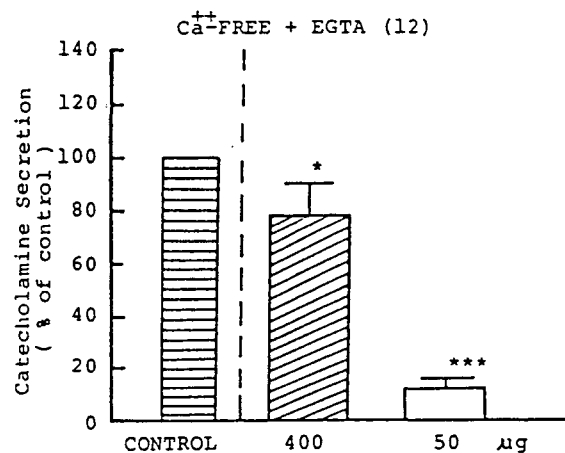


Fig. 8. Effect of perfusion with calcium-free Krebs solution plus EGTA on catecholamine-secretion evoked by Panaxadiol or acetylcholine. Secretion of catecholamine was evoked following perfusion of calcium-free Krebs solution containing  $5.0 \text{ mM}$ -EGTA for 30 min. Other legends are the same as in fig. 2 and 3. \*:  $P < 0.05$ . \*\*\*:  $P < 0.001$ .

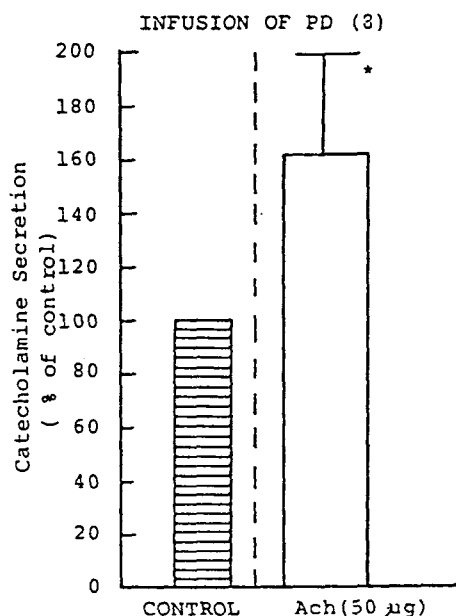


Fig. 9. Effect of infusion of Panaxadiol on acetylcholine-induced secretion of catecholamine from the rabbit adrenal gland. Secretion of catecholamine was evoked 30 min after perfusion of Krebs solution containing Panaxadiol(400µg/30 min) into the gland. Other legends are the same as in fig. 2 and 3.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

in adrenal gland. Therefore, it seems to be interesting to test whether PD will be able to enhance Ach-activity in CA secretion of CA. Ach-evoked secretion of CA after perfusion of Krebs solution containing PD(400µg/30min) for 30min. is shown in Fig.9. In 8 experiments, CA secretion induced by Ach in the presence of PD was enhanced significantly by  $169.4 \pm 41.2\%$  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 presynaptic 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 release 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 nicotinic receptors.

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

Furthermore, Brandt and his colleagues (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 acetylcholine release at the neuromuscular junction and in 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) also have 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. Adenosine 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 adenosine 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 indispensable role of calcium in the neurosecretory process has been thoroughly 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,  $10^{-6}$ M is level of intracellular calcium that gives half-maximal release of catecholamines 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  $\text{Ca}^{2+}$ -free Krebs solution, that evoked by PD was maintained at the level of about 75% of the control in zero  $\text{Ca}^{2+}$  medium. In the case of cat adrenal, Douglas and Rubin (1963) observed over 99% blockade of CA out flow in response to Ach in  $\text{Ca}^{2+}$ -free Locke solution. In perfused bovine adrenal gland (Phillips and Schuman, 1962) and bovine adrenal slices (Oka et al., 1965), a complete blockade of CA secretion by Ach in  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ -free Krebs solution 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 extracellular 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  $\text{Ca}^{2+}$ -free medium. Since PD promotes the release of CA by an extracellular calcium-

dependent process, the underlying secretory mechanism 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 ouabain (Schwartz, 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 releases CA from the perfused cat adrenal gland by a calcium-dependent exocytotic mechanism, which is due to a direct action on chromaffin cell itself, and that this secretory effect of CA evoked by ouabain is exerted through redistribution of monovalent 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 accumulation and potassium loss, and that such monovalent cation redistribution may cause a rise of intracellular ionized calcium level first, by depolarization of chromaffin cells or secretion, and by activation of the sodium-dependent calcium influx system. Moreover, it is found that ginseng inhibits  $\text{Na}^{+}$ ,  $\text{K}^{+}$ -ATPase in some biological systems (Kim et al., 1977; Chough et al., 1978; Kim et al., 1980; Lee et al., 1985).

It is well established that depolarization of the chromaffin cell produces and increase in calcium uptake (Douglas and Poisner, 1962), but it has also been shown that maintained depolarization of adrenergic neurons and chromaffin cells induces a sharp secretory response which rapidly 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 not shown) and this fact was dissimilar to that induced by ouabain (Garcia et al., 1980), which is a long-lasting and dose not apparently desensitize.

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F. Sandberg: Panaxadiol is the aglykon of a group of ginsenoside. Is it really water-soluble? How is it made into water-soluble for your experiment?

D. Y. Lim: Panaxatriol used in my experiment was very well dissolved in water. I got panaxatriol from Prof. Kim, Young-Ho, Department of Chemistry, Sejong University in Seoul.

H. Saito: Do you have some information on the effect panaxitriol on corticosterone secretion from adrenal gland?

D. Y. Lim: No. I don't have any information on that. But I will try it in next experiments.

S. C. Shim: I am wondering about the biological & physiological significance of your study. what will be the chance of panaxatriol getting into the adrenal gland when it is taken orally?

D. Y. Lim: My experimental results suggest that ingestion of large amounts or long-term ingestion of ginseng can cause hypertensive crisis, especially in the hypertensive patients by increasing the catecholamine secretion from the adrenal gland and that ginseng should be avoided in the hypertensive patients.

### 파낙스디올의 가토적출부신의 카테콜아민분비 작용에 관한 연구

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본 연구에서 한국산 인삼의 활성성분의 하나인 panaxadiol(PD)의 가토적출부신에서 카테콜아민(CA)의 분비작용과 작용기전을 파악하고자 실험을 시행하여 다음과 같은 결과를 얻었다. PD (400  $\mu\text{g}$ )을 가토적출부신에 투여 하였을 때 카테콜아민의 분비를 유의성 있게 증가시켰다. PD의 이러한 CA분비작용은 atropine 처리로 현저히 억제되었다. Physostigmine 처리시 PD뿐만 아니라 Ach의 CA 분비작용은 뚜렷이 증가 되었다. 그러나 chlorisondamine 처리로 PD나 Ach의 분비효과는 억제되었다. 또한 PD(400 $\mu\text{g}$ /30min)을 주입한 후에 Ach의 CA분비작용은 증강되었다. Ouabain에 의해서 PD의 작용은 약화되었으나 Ach의 CA분비효과는 오히려 강화되었다. PD나 Ach의 작용은 adenosine 전처리시 현저히 증강되었다. EDTA(5mM)와 함께 Ca-free Krebs액으로 30분 주입한 경우에 Ach의 분비작용은 거의 전적으로 차단되었으며, PD의 작용도 약화되었다. 이상의 실험결과로 보아, PD는 가토적출부신에서  $\text{Ca}^{+2}$  의존적으로 CA분비를 증가시키며, PD의 이러한 작용은 부신내의 cholinergic muscarinic 및 nicotinic receptor의 흥분작용에 기인되고, chromaffin cell에 대한 일부 직접작용에 의해서 나타나는 것으로 사료된다.