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# Influence of Panaxatriol-type Saponin on Secretion of Catecholamines from Isolated Perfused Rabbit Adrenal Gland

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**Abstract**□In the previous observations, it was reported that both total ginseng saponin and panaxadiol revealed the marked secretory effect of catecholamines (CA) from the rabbit adrenal gland and that CA secretion induced by them is due to dual mechanisms, cholinergic action and the direct action. In the present study, an attempt to investigate the effect of panaxatriol-type saponin (PT), which is known as an active component of Korean ginseng, on the secretion of CA from the rabbit adrenal gland was made. PT(200 $\mu$g) administered into adrenal vein evoked significantly secretion of CA from the isolated perfused rabbit adrenal gland. Secretory effect of CA produced by PT was attenuated clearly by treatment with chlorisondamine or adenosine, but was markedly increased by physostigmine. Perfusion of Krebs solution containing PT(200 $\mu$g) for 30 min potentiated greatly secretion of CA induced by acetylcholine. PT-induced CA secretion was weakened considerably by ouabain treatement or perfusion of calcium-free Krebs solution. These experimental data demonstrate that PT releases CA from the isolated perfused rabbit adrenal gland by a calcium-dependent exocytotic mechanism. It seems that the secretory effect of PT is caused through the release of acetylcholine from cholinergic terminals present in the adrenal gland and a direct action on the chromaffin cell itself.

**Keywords**□Panaxatriol-type saponin, catecholamine-secretion, adrenal gland, *Panax ginseng* C.A. Meyer.

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, the effort has been made to investigate the action of ginseng on blood pressure and to clarify the mechanism of pressor or depressor of ginseng in a series of experiments of pharmacological actions of it, especially on blood pressure.

Some investigators[1-6] found that ginseng caused hypotensive action, while others[7,8] reported its hypertensive action. However, Wood and his colleagues,[9] and Park[10] and Petkov[11] have shown that ginseng extract exerts a biphasic action on blood pressure, namely transient fall followed by prolonged elevation. Sokabe *et al.*[12] 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.

Recently, Lim *et al.*[13] also found that total ginseng saponin produced the pressor and the depressor action in the rat, and that its depressor response is exerted partly through the stmulation of cholinergic muscarinic receptors with the blockade or adrenergic alpha-receptors, and that the pressor response is caused by stimulation of nicotinic receptors in autonomic ganglia. Some investigators[14-16] reported that ginseng 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.

It has been reported that ginseng total saponin causes the increased secretion of catecholamines (CA) in the isolated perfused rabbit adrenal gland through stimulation of cholinergic muscarinic re-

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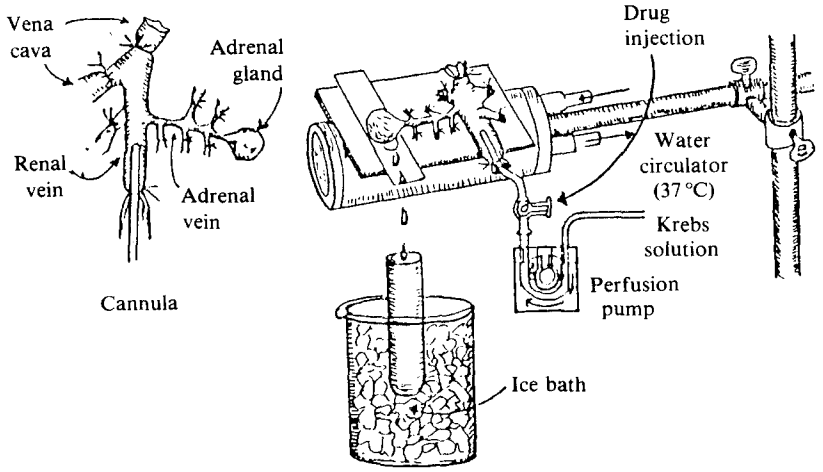


**Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamine in the isolated perfused adrenal gland of the rabbit.**

ceptors with partly direct action.[17]

More recently, Imamura and Washima[18] have suggested that Red ginseng may lower systolic blood pressure and may improve quality of life in hypertensive patients treated with antihypertensive agents.

In 1988, Lim *et al.*[19] has described that panaxadiol also produces secretion of CA from the isolated rabbit adrenal gland by calcium-dependent exocytotic mechanism, and that secretory effect may be due to the stimulation of cholinergic muscarinic and nicotinic receptors present in the adrenal gland and partly to a direct action on chromaffin cell itself. However, little is known about the mechanism of pressor action evoked by ginseng although there are many reports on the mechanism of its hypotensive activity as aforemetioned.

Therefore, in a series of experiments to clarify the effects of various components of ginseng on the response of blood pressure, especially pressor response, this study was designed to investigate the effect of panaxatriol-type saponin, one of chemically well-defined constituents of ginseng, on secretion of CA from the isolated rabbit adrenal glands and to elucidate the mechanism of its action.

## 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 adrenal gland was isolated by a modification of the method of Wakade.[20] The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractors. The stomach, intestine and portions of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and for cannultions.

As shown in Fig. 1, a cannula, used for perfusion of the adrenal gland(A), was inserted into the distal end of the renal vein after all the branches of the adrenal vein, the renal vein (if any), vena cava and aorta were ligated. Heparin (400 IU/m*l*) 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 sure that no leakage was present, and the perfusion fluid escaped only from the slit of the adrenal gland. Then the adrenal gland, along with the ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37 ± 1 °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

lsco pump at a rate of about 0.8 m*l*/min. The perfusion was carried out with Krebs bicarbonate solution of the following composition(mM): NaCl, 118.4; KCl, 4.7: $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; $KH_2PO_4$, 1.2; glucose, 11.7. The solution was bubbled with 95% $O_2$ + 5% $CO_2$ and the final pH was 7.4 ± 0.5. The solution contained disodium EDTA (10 $\mu$g/m*l*) and ascorbic acid (100 $\mu$g/m*l*) to prevent oxidation of catecholamine.

***Panaxatriol-type saponin (PT) and acetylcholine (Ach) injection***

200 $\mu$g of PT or 50 $\mu$g of Ach were injected in a volume of 0.05 m*l* into the perfusion stream via a three way stopcock (Fig. 1). In the preliminary experiments it was found that upon injection of the aforementioned doses of PT or Ach, the secretory response returned to preinjection level in about 4 min after administration of PT or Ach. Generally, the adrenal gland was perfused with Kreb's solution at least for more than an hour before stimulation. The adrenal perfuseate was collected in chilled tubes. Details of the collection of samples are given in the Results section.

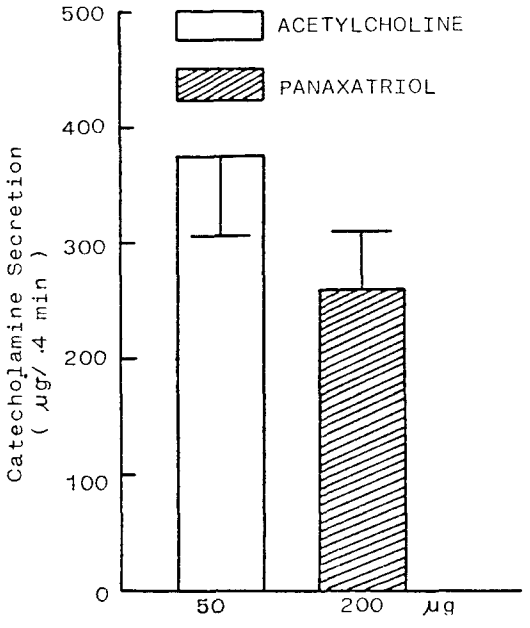


**Fig. 2. Release of catecholamines from the isolated perfused rabbit adrenal glands following injection of panaxatriol or acetylcholine.**

Adrenal glands are perfused with Krebs solution for 30 min, and then secretion of catecholamines was evoked by panaxatriol(200 $\mu$g) or Ach(50 $\mu$g) as shown. Each sample was collected for 4 min. Twelve adrenal glands were used in this experiment. Vertical lines over and below each column show S.E. of mean. Abscissa: doses of Ach or panaxatriol ($\mu$g), Ordinate: secretion of catecholamines.

***Analysis of Catecholamines***

CA content of perfusate was measured, directly by the fluorometric method of Anton and Sayre,[48] without the intermediate purification on alumina, using fluorospectrophotometer (Shimazu Co.). A volume of 0.2 m*l* of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by Ach or PT 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 octahydrate, EGTA (Sigma Chemical Co.), and chlorisondamine chloride (CIBA, Co.). PT was prepared from Panax ginsengs.

## RESULTS

***Catecholamines secretion in response to Acetylcholine or Panaxatriol-type saponin***

Secretion of CA evoked by PT 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 200$\mu$g-PT of 50$\mu$g-Ach into the perfusion stream caused significant secretion of great amounts of CA over the background secretion. The net CA secretion during 4 min in the case of 200 $\mu$g-PT was 259.2 ± 49.9 $\mu$g/min ($p < 0.001$), and in the case of injection with 50 $\mu$g-Ach, the release was 376.5 ± 75.5 $\mu$g/min of CA ($p < 0.001$). From our unpublished results, it was found that 200 $\mu$g-PT produced most significant secretory response of CA among 50, 100, 200, and 400 $\mu$g of PT used in the present study. Therefore, in all subsequent experiments a dose of 200 $\mu$g-PT was used with a dose of 50 $\mu$g-Ach in order to compare their results.

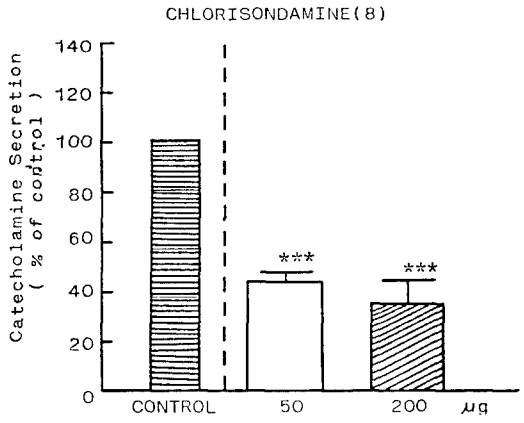


**Fig. 3. The effect of chlorisondamine on secretion of catecholamines evoked by panaxatriol or by acetylcholine.**

Catecholamines-secretion was produced 30 min following perfusion of the adrenal gland with Krebs solution containing 1.0 $\mu$M-chlorisondamine. Numeral in the above bracket shows number of animals used in this experiment. ***: $p<0.001$

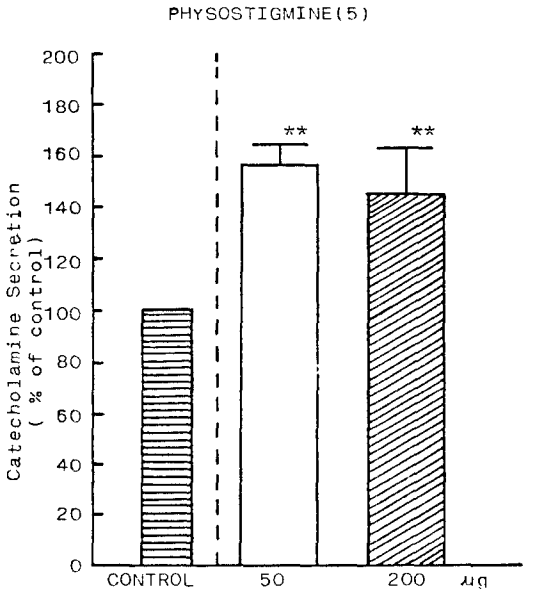


**Fig. 4. The effect of physostigmine on the release of catecholamines evoked by panaxatriol or by acetylcholine.**

Secretion of catecholamines was induced 30 min following perfusion of the adrenal gland with Krebs solution containing 10 nM-physostigmine. Other legends are the same as in Fig. 2 and 3. **: $p<0.01$

It was found that these results were similar to those of the study which used total ginseng saponin[17] or panaxadiol.[19]

***Effect of physostigmine on the release of catecholamines induced by Panaxatriol-type saponin***

Since PT could 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 PT and physostigmine,[25] which is an acetylcholinesterase inhibitor. When PT and Ach are introduced 30 min after the start of 10 nM-physostigmine treatment, CA outputs evoked with Ach (50 $\mu$g) and PT (200 $\mu$g) were potentiated markedly to $158.71 \pm 8.15\%$ ($p<0.01$) and $145.5 \pm 13.68\%$ ($p<0.01$) of the corresponding control value, respectively (Fig. 4).

***Effect of chlorisondamine on the release of catecholamines induced by Panaxatriol-type saponin***

In terms of the fact that PT-evoked CA release was potentiated by physostigmine, the CA secretion induced by PT could be secondary to the release of Ach produced by the drug from the presynaptic cholinergic nerve terminals present in the adrenal medulla. In each of 8 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.[25] CA secretion evoked by PT and Ach were markedly inhibited by $35.1 \pm 11.48\%$ ($p<0.001$) and $43.6 \pm 2.97\%$ ($p<0.001$), respectively, of the each corresponding control as shown in Fig. 3.

***Effect of infusion of Panaxatriol-type saponin on acetylcholine-induced secretion of catecholamines***

As aforementioned in Fig. 4 it was thought that PT may exert CA secretion by muscarine-like action in adrenal gland. Therefore, it seems to be interesting to test whether PT will be able to enhance Ach activity in secretion of CA. Ach-evoked secretion of CA after perfusion of Krebs solution containing PT(200 $\mu$g/30min) for 30 min is shown in Fig. 5. In 6 experiments, CA secretion induced by Ach in the presence of PT was enhanced significantly by $147.77 \pm 13.31\%$ of the control ($P < 0.02$).

***Effect of adenosine on catecholamines secretion evoked by Panaxatriol-type saponin or acetylcholine***

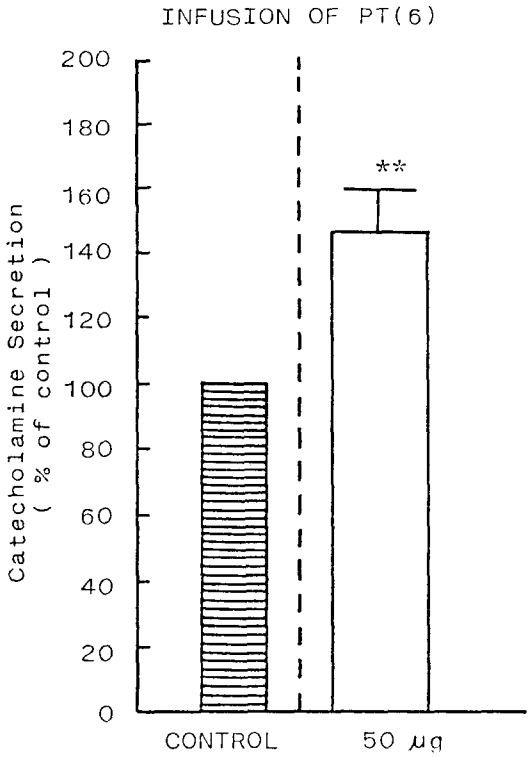


**Fig. 5. The effect of infusion of panaxatriol on secretion of catecholamines evoked by acetylcholine.**
Ach(50 $\mu$g) was given into perfusion stream after perfusion of panaxatriol(200 $\mu$g) for 30 min, and then sample was collected for 4 min. Other legends are the same as in Fig. 2 and 3. **: $p < 0.02$

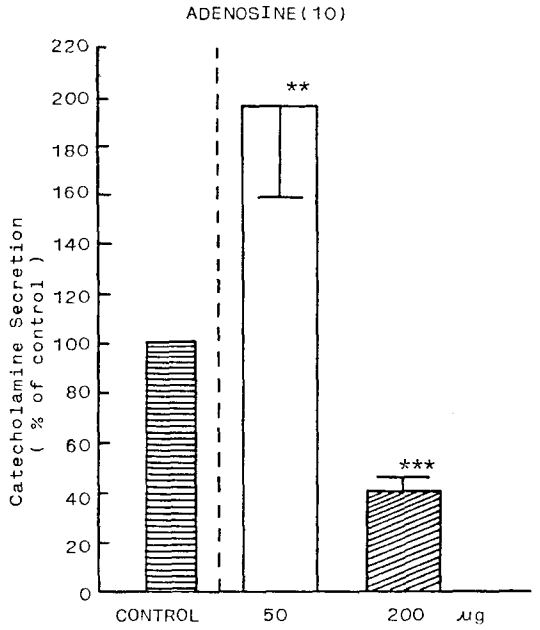


**Fig. 6. The effect of adenosine on catecholamines-secretion evoked by panaxatriol or by acetylcholine.**
The adrenal gland was perfused for 30 min with Krebs solution containing 0.18mM-adenosine and in the presence of adenosine secretion of catecholamines was produced by panaxatriol(200 $\mu$g) or by Ach(50 $\mu$g). Other legends are the same as in Fig. 2 and 3. **: $p<0.01$, ***: $p<0.001$

Since the data obtained in Fig. 3,4 and 5 indicated that CA secretion evoked by PT was induced partly by the action of Ach released through excitation of muscarinic and nicotinic receptors on chromaffin cells, it was therefore of particular interest to study the effect of adenosine on CA release evoked by PT or exogenous Ach. The results of these experiments are shown in Fig. 6. In each of 10 experiments, perfusion of the adrenal gland with 18 mM-adenosine for 30 min resulted in significant increases in CA secretion evoked by Ach which was $196.1 \pm 27.44\%$ ($p<0.01$) of the control, while CA output evoked by PT was significantly reduced by $40.7 \pm 5.42\%$ ($p<0.001$) of the control.

### *Effect of ouabain on catecholamines secretion induced by acetylcholine Panaxatriol-type saponin*

It has been demonstrated that cardiac glycosides induce the release of CA in the perfused bovine adrenal gland, guinea pig vas deferens, rabbit heart, cat spleen slices and the perfused cat adrenal gland. 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 PT or Ach in the rabbit adrenal gland. In 8 experiments, after obtaining control secretion, the adrenal gland was perfused with 2.0 $\mu$M-ouabain for 30 min and the secretory response was evoked in the presence of ouabain. As shown in Fig. 7, secretory response evoked by PT was depressed significantly by $22.5 \pm 8.7\%$ ($p<0.001$) of the control, while CA release induced by Ach was markedly potentiated by $200.5 \pm 78.20\%$ ($p<0.05$) of the control.

### *Effect of prolonged perfusion with $Ca^{++}$-free medium plus EGTA on Catecholamines secretion evoked by Panaxatriol-type saponin*

Since Dixon *et al.*[23] (1975) have shown that the physiological release of CA and dopamine beta-hydroxylase from the perfused cat adrenal gland is

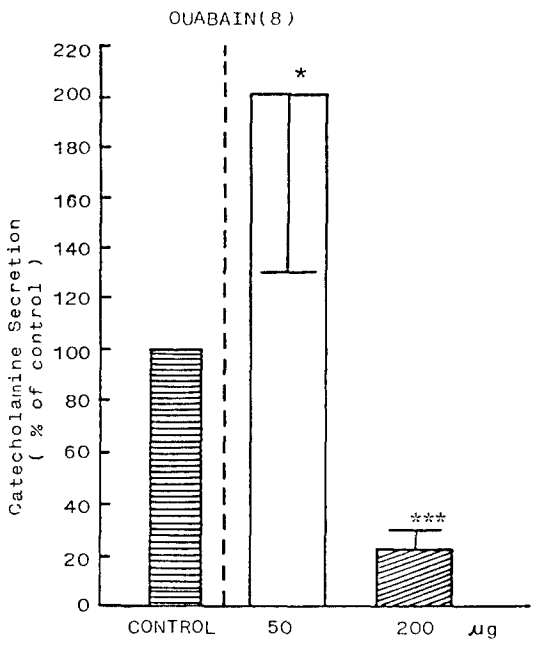


**Fig. 7. The effect of ouabain on secretion of catecholamines evoked by panaxatriol or by Ach.**
Catecholamines-secretion by panaxatriol was evoked 30 min after perfusion of Krebs solution containing $2 \times 10^{-6}$M-ouabain. Other legends are the same as mentioned in Fig. 2 and 3. *: $p<0.05$, ***: $p<0.001$

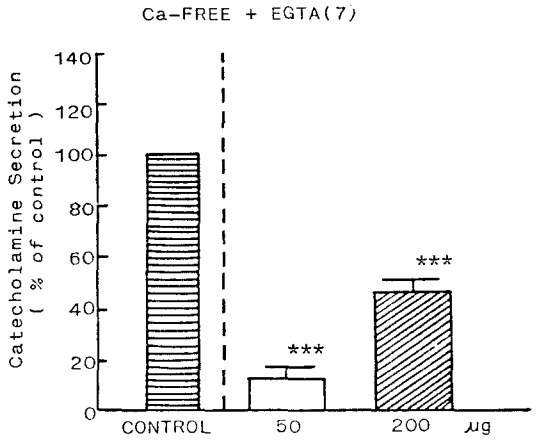


**Fig. 8. The influence of perfusion with Ca-free medium and EGTA on catecholamines-secretion evoked by panaxatriol or by Ach.**
In this experiment, after obtaining control response the glands were perfused for 30 min with 5mM-EGTA in Ca-free Krebs solution and the stimulated with panaxatriol(200 μg) or with Ach (50 μg). Other legends are the same as in Fig. 2. and 3. ***: $p<0.001$

dependent on the extracellular calcium concentration, it was of interest to examine whether the secretory effect induced by PT in this preparation of the rabbit was also related to extracellular calcium ions. Therefore, adrenal glands were perfused 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 PT or Ach. In 7 experiments, perfusion of gland with this solution for 30 min led to almost complete disappearance of the response evoked by Ach to $11.6 \pm 48\%$ ($p<0.001$) of control, while CA secreted by PT was depressed merely by $46.4 \pm 4.24\%$ ($p<0.001$) of the corresponding control value.

## DISCUSSION

The present works clearly demonstrate that PT produces an increased release of CA from the isolated perfused rabbit adrenal gland and that this secretory effect is critically dependent on the extracellular calcium concentration. It seems that this secretory effect evoked by PT may be due to the stimulation of cholinergic muscarinic and nicotinic receptors present in the adrenal gland and to partly a direct action on chromaffin cells.

Thus, the secretory effect of CA evoked by PT is very similar to that evoked by panaxadiol[19] or by ginseng total saponin[17] in our previous studies.

The adrenal medulla has been employed as a model system to study numerous cellular functions involving not only noradrenergic nerve cells but also neurons in general. One of such functions is neurosecretion. During the neurogenic stimulation of the adrenal medulla, Ach is released from the splanchnic nerve endings and activates cholinergic receptors on the chromaffin cell membrane.[21] This activation initates a series of events known as stimulus-secretion coupling, culminating in the exocytotic release of CA and other components of the secretory vesicles into the extracellular space. In general, two mechanisms are involved in the secretion of adrenal medullary hormones. Upon excitation of splanchnic nerves, Ach is released from the nerve terminals and then it activates nicotinic and muscarinic receptors of the chromaffin cells, causing exocytotic secretion of CA.

Ach, the physiological presynaptic transmitter at

the adrenal medulla, releases CA and dopamine-beta-hydroxylase by a calcium-dependent secretory process.[22-23] Since PT also induces the release of Ach from cholinergic nerve terminals in some other biological systems,[24] a question arises whether secretion of CA evoked by PT in the rabbit adenal gland is due secondarily to release of Ach from cholinergic terminals present in gland or to the direct action on adrenal medulla.

Since chlorisondamine, a well-known ganglionic blocking agent,[25] did not blocked completely the secretory responses of PT, it is felt that PT effect may be due partly to stimulation of muscarinic receptors or to the direct action on the chromaffin cells in addition to nicotinic action. However, inhibition of acetylcholinesterase with physostigmine[25] before the PT injection clearly enhanced the drug's secretory effect. This knotty contradictory finding can be explained if PT also releases Ach from presynaptic cholinergic sites, as it does in other cholinergic systems but the amounts of Ach being released are probably low and the Ach is quickly degraded by AchE before reaching the muscarinic and nicotinic receptors located on the surface of the chromaffin cell. Moreover, the finding that during perfusion of PT Ach-evoked secretion was markedly enhanced may support the potentiating effect of physostigmine to CA secretion evoked by PT. The fact that nicotinic(but not muscarinic) stimulation also releases soluble AchE from the chromaffin cells by a calcium-dependent mechanism[26,27] appears to be considerably related to these experimental results. Despite of a series of these facts, it is not likely to exclude that PT may have at least partly the direct action in CA secretion. Anyway, these observations strongly suggest that PT may produce the release of CA through the activation of muscarinic receptors with nicotinic receptors.

Douglas *et al.*[28] 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[29] also reported that atropine blocked the depolarizing effect of Ach in the rat adrenal chromaffin cells.

Adenosine reduced CA secretion evoked by PT of the adrenal gland but potentiated that evoked by exogenous Ach. These observations can be interpreted on the basis of differential electrical properties of pre-and postsynaptic membranes of the rabbit adrenal medulla. Generally, it is known that adenosine inhibits norepinephrine release from sympathetic neurons as well as acetylchoine release at the neuromuscular junction and ganglia,[30] and that in the brain, adenosine is also almost uniformly inhibitory in its action on neuronal firing.[31] Wakade and Wakade[20,32] 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. Adenosine thus could reduce Ach release, and thereby CA secretion.

In contrast with these reports, adenosine potentiated markedly CA secretion induced by Ach in the present experiment. In our previous study, it has been already found that adenosine enhances Ach-evoked secretory activity of CA in rabbits, and that this effect is through adenosine receptors located on chromaffin cells.[33] PT-evoked secretory effect of CA was reduced by adenosine-treatment as in the same manner to that evoked by transmural stimulation. It seems that the effect of PT may be associated with shortening the duration of nerve action potential.

The indispensible role of calcium in the neurosecretory process has been thoroughly established. According to the assumptions of Baker and Knight,[34,35] the relationship between concentration of intracellular calcium and transmitter release has not yet been determined in nerve terminals. As mentioned above, calcium plays the crucial role in many secretory mechanisms. Furthermore, there appears to be important parallels between depolarization-neurotransmitter release coupling in many other types of secretory cells.[36-38] In the present work, removal of extracellular $Ca^{++}$ depressed CA secretion evoked by PT or Ach. The secretory response evoked by Ach was almost extinguished in calcium-free Krebs solution containing 5 mM-EGTA, while that evoked by PT was maintained at the level of about 46.4% of the control secretion in zero $Ca^{++}$ medium. It has been shown that CA outflow in response to Ach are almost blocked by the exposure of $Ca^{++}$-free medium in various animal adrenal glands.[39-41]

In this experiment, the reason for the considerable response to PT in $Ca^{++}$-free Krebs solu-

tion is 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.[34] Such a store may not be easily depleted by removal of extracellular calcium. Some investigators,[42-44] reported that intracellular stores of calcium have been shown to play some role in contraction of smooth muscle produced by noradrenaline or Ach in $Ca^{2+}$-free medium. Since PT promotes the release of CA by 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 PT is achieved by a rise in the intracellular inonized calcium concentration. In support of such an idea, recently, Kao and Schneider[45] found that Ach evoked a large increase in cytosolic free-calcium in bovine chromaffin cells, most of which is blocked by hexamethonium treatment or removal of extracellular calcium, and that a small component of the Ach-evoked rise in cytosolic free $Ca^{++}$ is independent of extracellular calcium and is unaffected by atropine. These results suggested that muscarinic receptors regulate cytosolic calcium in chromaffin cells by a new mechanism different from that of nicotinic receptors, a mechanism utilizing an intracellular calcium source.

In the present study, ouabain enhanced CA secretion evoked by Ach but clearly depressed that evoked by PT. Since ouabain facilitated CA secretion evoked by exogenous Ach, it appears that effect of ouabain is mostly on the postsynaptic sites or the chromaffin cells of the adrenal gland. It is well-known that ouabain is a specific inhibitor of $Na,^{+}, K^{+}$-activated ATPase in many biological systems.[46,47] Garcia *et al.*[49] 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. Wakade[20] has shown that the mechanism responsible for producing the facilitation by ouabain is believed to be very similar to inhibition of $Na,^{+}, K^{+}$-ATPase for the enhancement of Ach release from presynaptic nerves in $K^{+}$-free medium. In terms of the fact that PT-perfusion enhanced greatly the secretory effect of CA evoked by Ach in the present work, it is felt that PT may produce CA secretion by the same mechanism with ouabain,[49] which also potentiates CA secretion evoked by Ach.

Furthermore, ginseng has been known to inhibit $Na^{+}$-$K^{+}$ ATPase in some biological systems.[50-53] Recently, Lee *et al.*[54] have described that ginseng saponins (triol < total saponin < diol) inhibited $Na,^{+}$ $K^{+}$-ATPase activity in a highly enriched preparation of cardiac sarcolemma prepared from dog ventricular myocardium. These facts suggest that the mechanism of PT-evoked CA secretion is very similar to that of ouabain-evoked CA secretion.

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