Influence of Total Ginseng Saponin on Catecholamine Secretion Evoked by Nicotinic Receptor Stimulation in the Perfused Rat Adrenal Gland

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Abstract

Lim and his coworkers (1987; 1988; 1989) have also found that all of total *Ginseng* saponin, panaxadiol- and panaxatriol-type saponins cause the increased secretion of catecholamines (CA) in a Ca^{2+}-dependent fashion from the isolated perfused rabbit adrenal glands through the activation of cholinergic (both nicotinic and muscarinic) receptors. These CA secretory effects are partly due to the direct action on the rabbit adrenomedullary chromaffin cells. However, the present study was designed to examine the effect of total ginseng saponin on CA secretion evoked by activation of cholinergic nicotinic receptors in the isolated perfused model of the rat adrenal gland.

Total ginseng saponin given (100 μg/20 min) into an adrenal vein did fail to produce alteration of spontaneous CA release from the rat adrenal medulla. Acetylcholine(5.32 mM)- and DMPP (100 μM, a selective nicotinic receptor agonist)-evoked CA secretory responses were reduced markedly after the pretreatment with the total ginseng saponin at a rate of 100 μg/6.2 ml/20 min, respectively. Pretreatment with total ginseng saponin also depressed greatly high potassium (56 mM, a membrane depolarizing agent)- and Bay-K-8644 (10 μM, a calcium channel activator)-induced CA secretions.

Taken together, it is thought that total ginseng saponin can inhibit the releasing effect of CA evoked by nicotinic receptor stimulation from the isolated perfused rat adrenal medulla, which seems to be associated to the direct inhibition of influx through L-type calcium channel into the rat adrenomedullary chromaffin cells. It seems that there is species differences in the adrenomedullary catecholamine secretion between the rabbit and rat.

Introduction

It has been known that *Ginseng* extract causes the hypotensive action (Hsu, 1956; Ozaki et al, 1963; Oh etal, 1968; Lee & Cho, 1971; Lee, 1974) while it rather produces the hypertensive action (Kitagawa & Iwaki, 1963; Siegel, 1979). Some studies have suggested that *Ginseng* extract causes a biphasic response on blood pressure, namely, transient fall followed by prolonged elevation (Park, 1960; Petkov, 1961; Wood et al, 1964). Total *Ginseng* saponin is found to produce the pressor and depressor actions in the anesthetized normotensive rats (Lim et al 1987). It has suggested that this depressor response is mediated in part through the blockade of adrenergic -receptors as well as the stimulation of cholinergic muscarinic receptors, and that its pressor response is caused by stimulation so nicotinic cholinergic receptors at the sympathetic ganglia.

Furthermore, *Ginseng*, when given at small dose in spontaneously hypertensive rat (SHR), causes pressor response, but at relatively large dose rather produces dose-dependent hypotensive response with decreased plasma renin activity (Shon et al, 1979; 1980; Seok et al, 1981). Sokabe and his coworkers (1984) have shown that administration of Korean Red *Ginseng* powder for 11 weeks has no effect on blood pressure in nor motensive Donryu (DON) rats, SHR and renal hypertensive rats, whereas it elevates slightly blood pressure in deoxycorticosterone salt hypertensive rats.

Lim and his coworkers (1987; 1988; 1989) have also found that all of total *Ginseng* saponin, panaxadiol and panaxatriol cause the increased secretion of catecholamines (CA) in a Ca^{2+}-dependent fashion from the isolated perfused rabbit adrenal glands through the activation of cholinergic (both nicotinic and muscarinic) receptors and partly the direct action on the rat adrenomedullary chromaffin cells.

On the mechanism of *Ginseng* induced hypertensive action, there are many reports, including serotonin antagonism, Ca^{2+}-antagonism, histamine release and the direct action on vascular smooth muscle, etc. However, there is not much evidence available to establish the mechanism of hypotensive action so far, and also much disagreement among the pharmacological effects of *Ginseng* especially on blood pressure depending on authors or its constituents.

Therefore, the present study was attempted to examine the effect of total *Ginseng* saponin on CA secretory responses evoked by stimulation of cholinergic nicotinic receptors in the isolated perfused rat adrenal gland and to clarify the mechanism of its action.

Materials and Methods

Experimental Procedure

Male Sprague-Dawley rats, weighing 150 to 350 grams, were anesthetized with ether. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractor. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

A cannula, used for perfusion of the adrenal gland (Fig. 1-A), was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Heparin (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 entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at

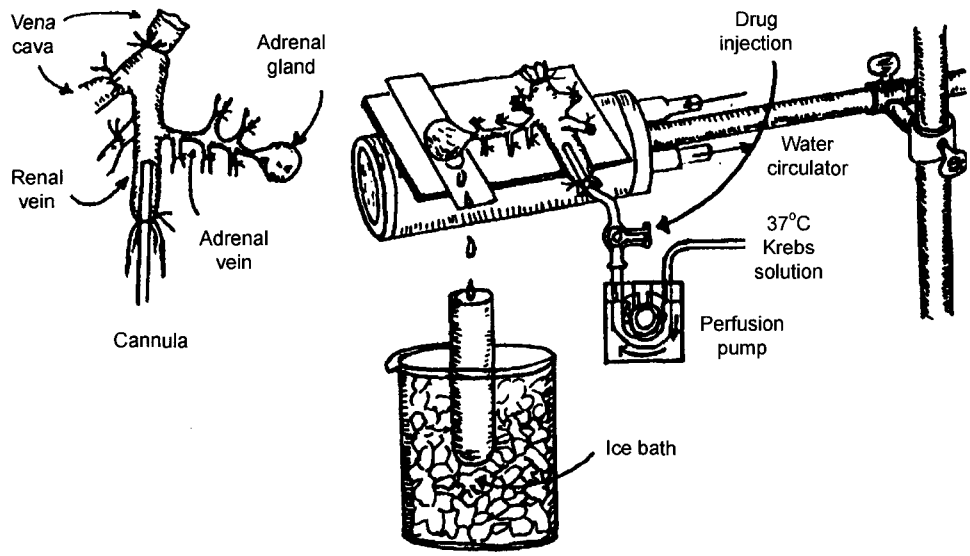

Fig. 1. Schematic drawing of the preparation used to study secretion of catecholamines in the isolated perfused rat adrenal gland.

37±1°C (Fig. 1-B)

Perfusion of adrenal gland

The adrenal glands were perfused by means of a ISCO pump (WIZ Co.) at a rate of 0.3 ml/min. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95% O_2 + 5% CO_2 and the final pH of the solution was maintained at 7.4 ± 0.05. The solution contained disodium EDTA (10 μg/ml) and ascorbic acid (100 μg/ml) to prevent oxidation of catecholamine.

Drug administration

The perfusions of DMPP (100 uM) for 2 minutes and/or a single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 ml were made into perfusion stream via a three-way stop-cock, and Bay-K-8644 (10^{-5} M) was also perfused for 4 min.

In the preliminary experiments it was found that upon administration of the above drugs, secretory responses to ACh, KCl, and Bay-K-8644 returned to pre-injection level in about 4 min, but the responses to DMPP in 8 min.

Collection of perfusate

As a rule, prior to stimulation with cholinergic agonists or excess K^+, perfusate was collected for 4 min to determine the spontaneous secretion of CA (background sample). Immediately after the collection of the backgound sample, collection of the perfusates was continued in another tube as soon as the perfusion medium containing the stimulatory agent reached the adrenal gland. Stimulated sample's was collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from those secrected from the stimulated sample to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effects of total *Ginseng* saponin on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing total *Ginseng* saponin for 20 min, then the perfusate was collected for a certain (background sample), and then the medium was changed to the one containing the stimulating agent and the perfusates were collected for the same period as that for the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (1962) without the intermediate purification with alumina for the reasons described earlier (Wakade, 1981) using fluorospectrophotometer (Shimadzu Co. Japan/ Kontron, Italy).

A volume of 0.2 ml of the perfusate was used for the reaction. The CA content in the perfusate of stimulated glands by secretogogues used in the present work was high enough to obtain readings several fold greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples. The content of CA in the perfusate was expressed in terms of norepinephrine (base) equivalents.

Statitical analysis

All data are presented as means with their standard errors, and the significance of differences were analyzed by Student's paired t-test using the computer program of statistics system as previously described (Tallarida and Murray, 1987).

Drugs and their sources

The following drugs were used: acetylcholine chloride, 1.1-dimethyl-4- phenyl piperazinium iodide [DMPP], norepinephrine bitartrate, and methyl-1, 4-dihydro-2, 6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl-pyridine-5-carbox-ylate [BAY-K-8644] (Sigma Chemical Co., U.S.A.). Total *Ginseng* saponin was a gift from late Dr. Young-Ho Kim (Sejong University, Seoul, Korea). Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644 was dissolved in 99.5% ethanol and diluted appropriately (final concentration of alcohol was less than 0.1%). Concentrations of all drugs used are expressed in terms of molar base.

Results

The effect of total Ginseng saponin on acetylcholine- and DMPP-evoked secretory response of CA from the isolated perfused rat adrenal gland

The resting (basal) CA secretion from the perfused rat adrenal glands reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 60 min before the experimental protocol is initiated. The basal release of CA amounted to 21.5±2.3 ng/2 min (n=10). The

effects of total *Ginseng* saponin on acetylcholine- as well as DMPP-mediated CA secretion from perfused rat adrenal glands were examined. In the present study, total *Ginseng* saponin itself did not produce any effect on basal CA output (data not shown).

When ACh (5.32 mM) in a volume of 0.05 ml was injected into the perfusion stream, the

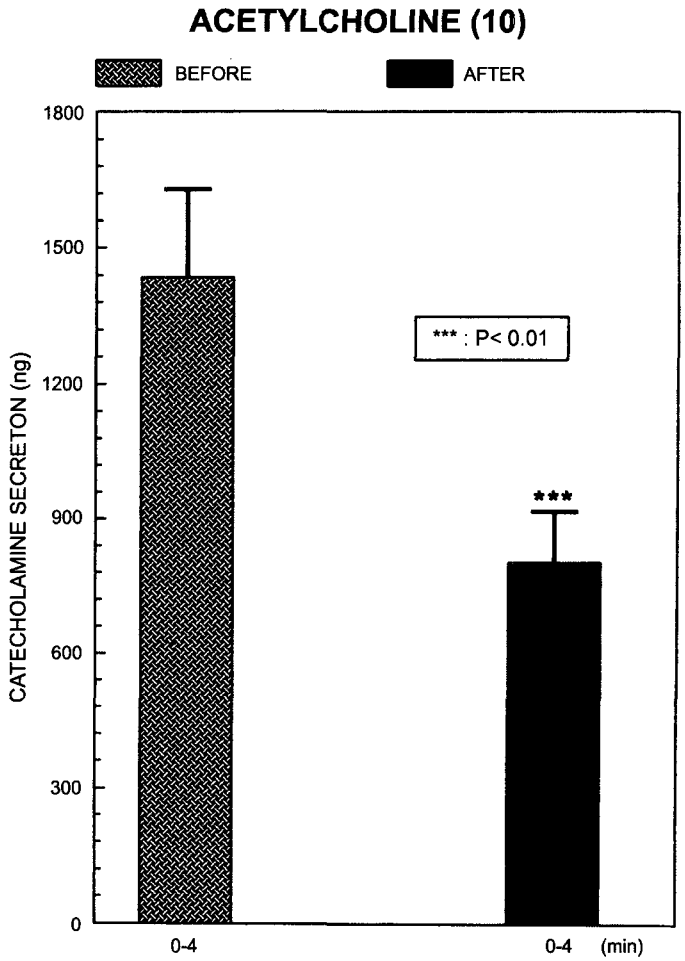

Fig. 2. Influence of total Ginseng saponin (GTS) on acetylcholine (ACh)-stimulated secretion of catecholamines (CA) from the isolated perfused rat adrenal glands. CA secretion was induced by a single injection of ACh (50 mg) after perfusion with normal Krebs solution for one hour prior to initiation of the experimental protocol. "CONTROL" and "AFTER" denote CA secretion evoked by ACh before (CONTROL) and after preloading with total ginseng saponin (100 mg/20 min), respectively. Number in the parenthesis indicates number of experimental rat adrenal glands. Vertical bars represent the standard error of the mean (S.E.M.). Ordinate: the CA secreted from the adrenal gland (ng/4 min). Abscissa: ACh (5.32 mM). Statistical difference was obtained by comparing the control with the pretreated group. Each perfusate was collected for 4 minutes. ***: P< 0.01.

amounts of CA secreted was 1435±194 ng for 4 min. However, after the preperfusion with total *Ginseng* saponin at a rate of 100 μg/6.2 ml/20 min ACh-stimulated CA secretion was significantly decreased to 804±113 ng ($P< 0.01$,) for 4 min from 10 adrenal glands as shown in Fig. 2.

When perfused through the rat adrenal gland, DMPP (100 μM for 2 min), which is a selective nicotinic receptor agonist in autonomic sympathetic ganglia, evoked a sharp and rapid increase in CA secretion. As shown in Fig. 3, DMPP-stimulated CA secretion before preloading with total *Ginseng* saponin was 2031±304 ng (0-4 min) and 391±72 ng (4-8 min), while after pretreatment with total *Ginseng* saponin at a rate of 100 μg/6.2 ml/20 min they were greatly reduced to

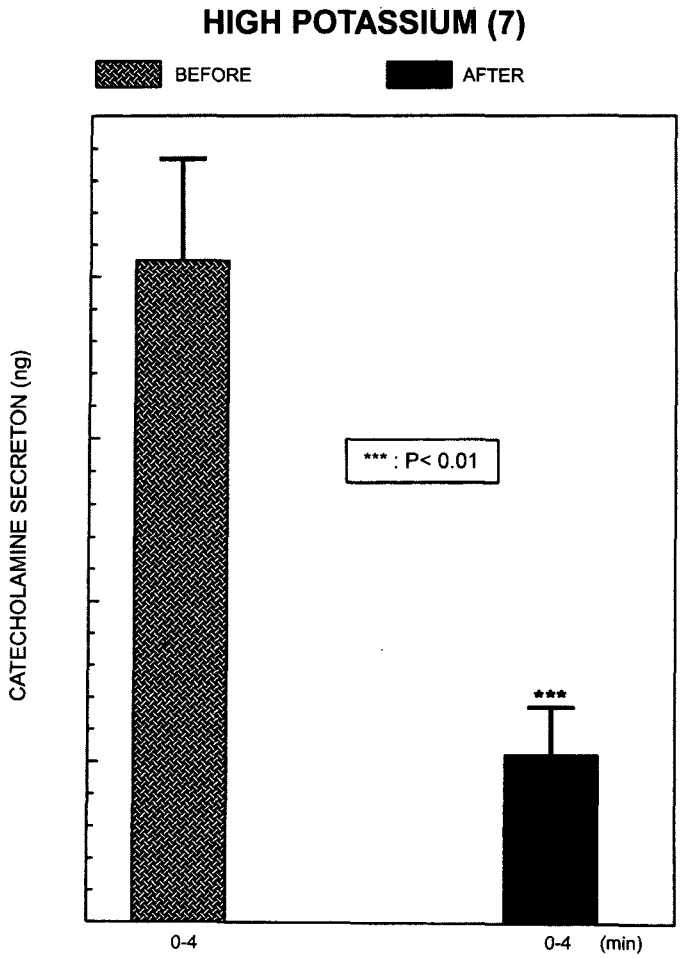

Fig. 3. Influence of total Ginseng saponin (GTS) on DMPP-stimulated catecholamine (CA) secretion from the isolated perfused rat adrenal glands. DMPP (100 mM) was induced for 2 min before and after preloading with GTS (100 mg/20 min). The perfusate was collected twice at 4 min-interval. Other legends are the same as in Fig. 2. ***: $P< 0.01$

964±83 ng (0-4 min, P< 0.01) and 121±21 ng (4-8 min, P< 0.01), respectively from 10 rat adrenal glands.

The effect of total Ginseng saponin on high K^+- and Bay-K-8644-evoked secretory response of CA from the isolated perfused rat adrenal gland.

High K exerts two distinct effectso cells: (1) depolarization of cell membrane, and (2) depolarization-induced influx of calcim via voltage-dependent calcium channels (Wada et al, 1985). In 7 adrenal glands, It was also found that high K^+ (56 mM) stimulated CA secretion, which was

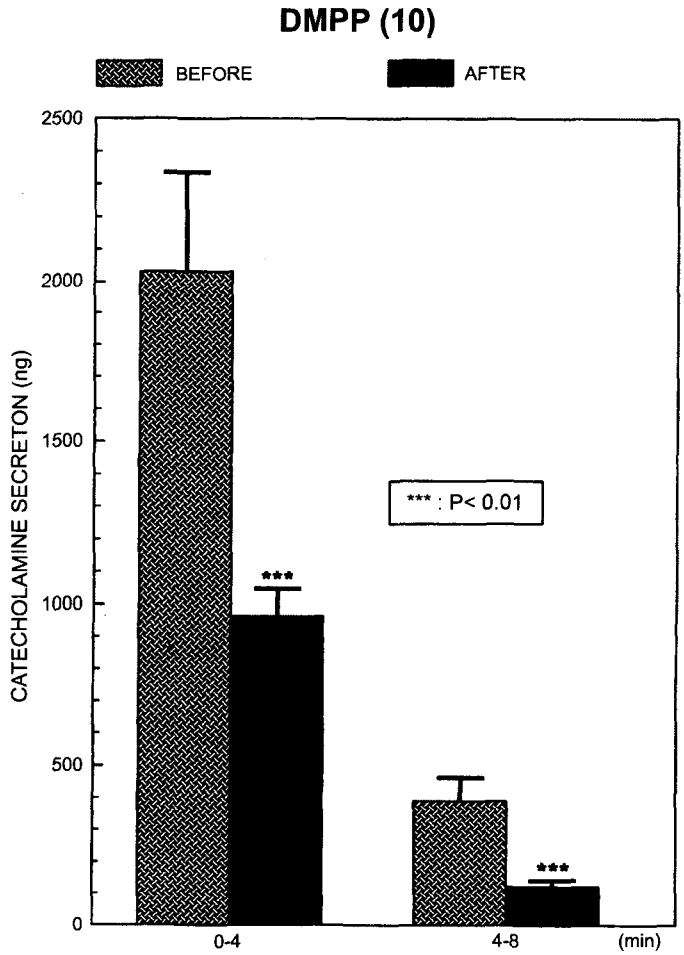

Fig. 4. Influence of total Ginseng saponin (GTS) on high potassium-stimulated catecholamine (CA) secretion from the isolated perfused rat adrenal glands. High potassium (56 mM) was induced in a volume of 50 ml before and after preloading with GTS (100 mg/20 min). Other legends are the same as in Fig. 2. ***: P< 0.01

attenuated significantly after the pretreatment with total *Ginseng* saponin at a rate of 100 ug/6.2 ml/20 min. In the presence of total *Ginseng* saponin, its CA secretion was 316±88 ng/4 min ($P< 0.01$), which was significantly lower than the control secretion (1231±191 ng/4 min) (Fig. 4).

Since Bay-K-8644 is known to be a calcium channel activator which causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm et al., 1983; Wada et al., 1985) and enhances basal Ca^{++} uptake (Garcia et al., 1984) and CA release (Artalejo & Garcia, 1986; Lim et al., 1992), it was of interest to determine the effects of total *Ginseng* saponin on Bay-K-8644-stimulated CA secretion from the isolated perfused rat adrenal glands. Fig. 5 illus-

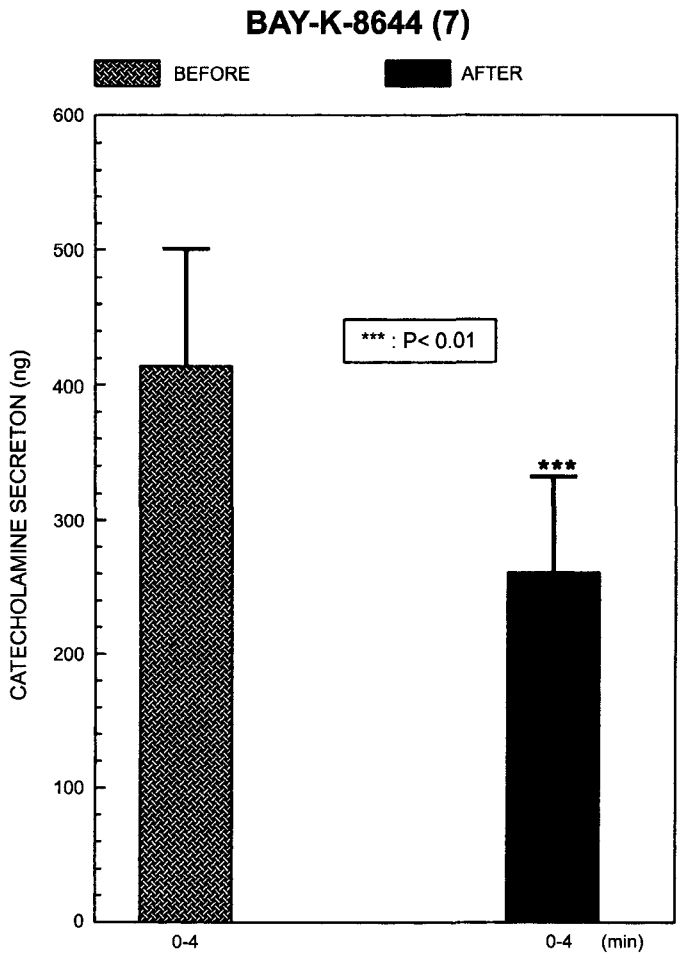

Fig. 5. Influence of total Ginseng saponin (GTS) on Bay-K-8644-evoked catecholamine (CA) secretion from the isolated perfused rat adrenal glands. Bay-K-8644 (10 mM) was induced for 4 min before and after preloading with GTS (100 mg/20 min). Other legends are the same as in Fig. 2. ***: $P< 0.01$

trates the inhibitory effect of total *Ginseng* saponin on Bay-K-8644-evoked CA secretion. Bay-K-8644 (10 μM) given into the perfusion stream for 4min before total *Ginseng* saponin increased CA secretion to 414±87 ng/4 min in 7 rat adrenal glands. However, under the effect of preloaded-ing with total *Ginseng* saponin at a rate of 100 μg/6.2 ml/20 min before Bay-K-8644 was introduced, Bay-K-8644-stimulated CA secretion was strikingly depressed to 261±71 ng ($P< 0.01$) for 4 min as compared to the corresponding control release; thus, the release was reduced to 62% of the control secretion.

Discussion

The present experimental results have shown that total *Ginseng* saponin inhibits greatly CA secretory responses evoked by ACh, DMPP, high potassium and Bay-K-8644 from the isolated perfused rat adrenal gland, and that this inhibitory effect may be exterted through the direct inhibition of calcium influx into the rat adrenomedullary chromaffin cells. Thus, the present findings appeared to contribute at least partly to the facts that *Ginseng* extract causes the hypotensive action (Hsu, 1956; Ozaki et al, 1963; Oh etal, 1968; Lee & Cho, 1971; Lee, 1974), but not to the fact that it rather produces the hypertensive action (Kitagawa & Iwaki, 1963; Siegel, 1979).

In general, the adrenal medulla has been employed as a model system to study numerous cellular functions involving not only noradrenergic nerve cells but also neurons. During neurogenic stimulation of the adrenal medulla, ACh is released from splanchnic nerve endings and activated chlonergic 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 CA and other components of the secretory vesicles into the extracellular space. Usually, two mechanisms are involved in the secretion of adrenal medullary hormons. Upon excitation of splanchnic nerves, ACh is released from the nerve terminals, and then is activates nicotinic secretion of CA.

In the present study, the findings that total *Ginseng* saponin inhibited the CA secretory responses evoked by nicotinic receptor stimulation as well as by membrane depolarization in the rat adrenal medulla seem to be able to support the fact that components of *Ginseng* saponins (panaxadiol and panaxatriol) causes vasodilatation through Ca^{++} antagonism in the isolated rabbit aorta (Lee, 1974).

These experimental results indicate that *Ginseng*-induced inhibitory activity of CA secretory

response evoked by stimulation of nicotinic receptors might contribute at least partly to its hypotensive mechanism. ACh, the physiological presynaptic transmittor at the adrenal medulla, which is released by depolarizing splanchnic nerve terminals and then activates nicotinic receptors, releases CA and dopamine -hydroxylase by calcium dependent secretory process (Dixon et al, 1975; Viveros et al, 1968).

In the light of this fact, the present results suggest that total *Ginseng* saponin may inhibit CA secretion evoked by nicotinic stimulation from the splanchnic nerve ending through the blockade of nicotinic receptors. Moreover, CA secretion evoked by DMPP, a selective nicotinic receptor agonist, was also attenuated by pretreatment with total *Ginseng* saponin. DMPP is a synthetic quaternary ammonium compound that is more selective for ganglionic receptors as a typical autonomic ganglionic stimulant (Rang and Dale, 1987). It also causes the hypertensive responses mediated by nicotinic receptors in cats, dogs and rats, which disappear after the blockade of adrenergic receptors and the autonimic ganglia (Chen et al, 1951). The releas of epinephrine from the adrenal medulla in response to splanchnic nerve stimulation or nicotinic agonist is mediated by activation of nicotinic receptors located on the chromaffin cells. The exocytotic CA release from the chromaffin cells appears to be essentially similar to that occuring in noradrenergic axons (Douglas, 1968; Sorimachi & Yoshida, 1979).

ACh-evoked CA secretion has been shown to be caused through stimulation of both nicotinic and muscarinic receptors in guinea-pig adrenal gland (Nakazato et al, 1988) as well asin the perfused rat adrenal glands (Lim & Hwang, 1991).

In the present study, total *Ginseng* saponin also depressed greatly CA secretory response evoked by Bay-K-8644, which is known to activate L-type voltage-dependent Ca^{2+} channels (Garcia et al, 1984; Schramin et al, 1983). This result indicates that total *Ginseng* saponin may inhibits Ca^{2+} influx to the rat adreno medullary cells.

In support of this idea, in cultured bovine adrenal medullary cells, nicotinic (but not muscarinic) receptors mediate the Ca^{2+}-dependent secretion of CA (Fish, Holz & Agronoff, 1981; Yanagihara et al, 1979). It has been also known that the activation of nicotinic receptors stimulates CA secretion by incresing Ca^{2+} entry through receptor-linked and/or voltage-dependent Ca^{2+} channels in both perfused rat adrenal glands (Wakade & Wakade, 1983; Lim & Hwang, 1991) and isolated bovine adrenal chromaffin cells (Kilpatrick et al, 1981; 1982; Knight & Kesteven, 1983). Wada and his coworkers (1985) have found that the adrenomedullary chromaffin cells have (i) nicotinic receptor-associated ionic channels, responsible for carbachol-induced Na

influx (ii) voltage-dependent Na channels, responsible for veratridine-induced Na^+ influx and (iii) voltage-dependent Ca^{2+} channels, suggesting that the influx of Na caused either by carbachol or by veratridine leads to activate voltage-dependent Ca^{2+} channels by altering membrane potentials, whereas high K^+ directly activates voltage-dependent Ca^{2+} channels without increasing Na influx. In the present study, the finding that high potassium-induced CA secretory response was markedly depressed by pretreatment with total *Ginseng* saponin indicates strongly that this inhibitary effect of total *Ginseng* saponin is exerted through the direct inhibition of calcium influx into the rat adrenal chromaffin cells. Furthermore, slight elevation in the extracellular potassium concentratio increases both the frequencey of spontaneous action potentials and the secretion of CA (Kidokoro & Ritchie, 1980), suggesting that the influx of calcium that occurs during action potentials is directly linked to the rate of secretion.

In conclusion, the present study using the isolated perfused rat adrenal glands suggested that total *Ginseng* saponin inhibits CA secretions evoked by stimulation of cholinergic nicotinic receptors as well as by membrane depolarization, resulting in the direct inhibition of calcium influx into the adrenomedullary chromaffin cells possibly through voltage-dependent membrane calcium channels. These experimental results may contribute, in part, to the hypotensive effect of *Ginseng* components, through inhibition of CA secretion from adrenal medullary chromaffin cells and consequent reduction of the CA level in the circulation.

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