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Green Tea Extract, not Epigallocatechin gallate Inhibits Catecholamine Release From the Rat Adrenal Medulla

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Abstract–The present study was designed to investigate the effects of green tea extract (CUMC6335) and epigallocatechin gallate (EGCG) on secretion of catecholamines (CA) in the isolated perfused rat adrenal gland. In the presence of CUMC6335 (100 μg/mL) into an adrenal vein for 60 min, CA secretory responses evoked by ACh (5.32 mM), high K^+ (56 mM) and Bay-K-8644 (10 μM for 4 min) from the isolated perfused rat adrenal glands were greatly inhibited in a time-dependent fashion. However, EGCG (8 μg/mL) did not affect CA release evoked by ACh, high K^+ and Bay-K-8644. CUMC6335 itself did fail to affect basal catecholamine output. Taken together, these results demonstrate that CUMC6335 inhibits greatly CA secretion evoked by stimulation of cholinergic nicotinic receptors as well as by the direct membrane depolarization from the isolated perfused rat adrenal gland. It is felt that this inhibitory effect of CUMC6335 may be due to blocking action of the L-type dihydropyridine calcium channels in the rat adrenal medullary chromaffin cells, which is relevant to the cholinergic nicotinic blockade. It seems that there is a big difference in mode of action between CUMC6335 and EGCG.

Keywords □ green tea extract (CUMC6335); epigallocatechin gallate; catecholamine release, blockade of nicotinic receptors

INTRODUCTION

Green tea, drink brewed from the dried leaves of *Thea sinensis (Theaceae)*, is the most frequently consumed beverage in the world apart from water (Graham, 1992) and has a long history of use having originated in China some 5000 years ago (Shalleck, 1981). Some epidemiological studies have suggested that both tea and flavonoids that can be derived from green tea may protect against cardiovascular disease (Hertog *et al.*, 1993; Keli *et al.*, 1996). Therefore, the physiological effects of tea and its components on cardiovascular disease risk factors such as blood pressure are of interest. Tea, which contains caffeine at about 3% of dry weight and polyphenolic compounds at about 40% of dry weight (Harbowy and Ballentine, 1997), makes a potentially significant contribution to the total intake of caffeine and polyphenolics including flavonoids. Despite this, there has been remarkably little research on the effects of tea ingestion on blood pressure.

Ingestion of caffeine results in a transient increase in blood pressure in subjects who have avoided caffeine for 12 h or more (Sung *et al.*, 1994; Pincomb *et al.*, 1996). Ingesting caffeine-containing tea also induces a transient increase in blood pressure (Quinlan *et al.*, 1997). However, extracts of tea (Fitzpatrick *et al.*, 1992) and flavonoids found in tea (Fitzpatrick *et al.*, 1993) have been shown to give vasodilator effects *in vitro*. The results of the few studies investigating the relationship between regular tea consumption and blood pressure have been inconsistent (Stensvold *et al.*, 1992; Bingham *et al.*, 1997; Rakic *et al.*, 1996; Abe *et al.*, 1995; Yokozawa *et al.*, 1994). In a cohort of Norwegian men and women, higher consumption of black tea was associated with lower systolic blood pressure (SBP) (Stensvold *et al.*, 1992). However, in a 4-week randomized, controlled, crossover trial in normotensive men and women, drinking six mugs of tea daily had no significant effect on clinic measured blood pressure (Bingham *et al.*, 1997). Moreover, in older treated hypertensive subjects, the postprandial falls in SBP were attenuated by tea consumption (Rakic *et al.*, 1996), although no significant alteration in 24-h ambulatory blood pressure was observed; this outcome was possibly related to the acute pressor effects of caffeine. The effects of green tea on blood pressure have not been examined in humans. Moreover, it has been shown that (–) epicatechin also reduced arterial con-

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traction induced by other vasoconstrictors, such as phenylephrine and endothelin-1 (Huang *et al.*, 1998). Recently, it has been also found that (−) epicatechin could act on endothelium to increase intracellular Ca^{2+} and nitric oxide release, which may account for the endothelium-dependent relaxation (Huang *et al.*, 1999) in rat isolated mesenteric arteries.

It has been suggested that oxidative stress is involved in the development of raised blood pressure (Romero-Alvia *et al.*, 1996), possibly via its effects on endothelial function (Briner and Luscher, 1994; Ferro and Webb, 1997; Flavahan, 1992). The main hypothesis tested in the two studies reported in this paper is that antioxidant (Rice-Evans *et al.*, 1995) and vasodilatory (Fitzpatrick *et al.*, 1993; Fitzpatrick *et al.*, 1992) polyphenolics in tea can attenuate the transient pressor effect of caffeine, and lower blood pressure during regular consumption. Recently, it has been also reported that pycnogenol stimulates constitutive endothelial NO synthase (eNOS) activity to increase NO levels, which could counteract the vasoconstrictor effects of epinephrine and norepinephrine (Fitzpatrick *et al.*, 1998). In contrast to these results, it has been shown that tea ingestion in the normotensive men caused larger acute increases in blood pressure than caffeine alone. However, any acute effects of tea on blood pressure did not translate into significant alterations in ambulatory blood pressure during regular tea (Hodgson *et al.*, 1999). Despite this, there has been remarkably little research on the effects of tea ingestion on blood pressure. More recently, Katayama and his co-workers (2002) have shown that EGCG can facilitate the cholinergic ganglion transmission possibly by increasing the amount of ACh released and, together with depolarizing action on myenteric neurons, may modulate the activity of the myenteric plexus of the guinea-pig ileum.

Therefore, the present study was attempted to examine the effects of CUMC6335 on catecholamine secretion from the isolated perfused model of the rat adrenal gland, and to compare its effect with that of epigallocatechin gallate.

MATERIALS AND METHODS

Experimental Procedure

Male Sprague-Dawley rats, weighing 180 to 250 grams, were anesthetized intraperitoneally with thiopental sodium (40 mg/kg). 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 the placement of three-hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads, and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations. A cannula, used for perfusion of the adrenal gland, was inserted into the distal end of the renal vein after all branches of adrenal vein (if any), vena cava and aorta were ligated. Before ligating vessels and cannulations, heparin (400 IU/mL) was injected into vena cava to prevent blood coagulation. 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. The adrenal gland, along with ligated blood vessels and the cannula, was then 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.

Perfusion of Adrenal Gland

The adrenal glands were perfused by means of a peristaltic 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 pH of the solution was maintained at 7.4~7.5. The solution contained disodium EDTA (10 μg/mL) and ascorbic acid (100 μg/mL) to prevent oxidation of CAs.

Drug Administration

The perfusion of Bay-K-8644 (10 μM) for 4 minutes was made into perfusion stream, respectively. A single injection of ACh (5.32 mM) and KCl (56 mM) in a volume of 0.05 mL was injected into perfusion stream via a three-way stopcock, respectively.

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.

Collection of Perfusate

Prior to stimulation with various secretagogues, perfusate was routinely collected for 4 min to determine spontaneous secretion of CA (background sample). Immediately after the collection of the background sample, the perfusates were continuously collected in another tube as soon as the perfusion

medium containing the stimulatory agent reached the adrenal gland. Stimulated samples were collected for 4 to 8 min. The amounts secreted in the background sample have been subtracted from that secreted 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 CUMC6335 on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing CUMC6335 for 60 min immediately after the perfusate was collected for a certain minute (background sample). And the medium was then 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 fluorospectrophotometrically (Kontron Co. Italy) measured directly by the fluorometric method of Anton and Sayre (1962) without intermediate purification on alumina for the reasons described earlier (Wakade, 1981).

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

Statistical Analysis

The statistical significance between groups was determined by utilizing the Student's t-test. A P-value of less than 0.05 was considered to represent statistically significant changes, unless specifically noted in the text. Values given in the text refer to means and standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computer program described by Tallarida and Murray (1987).

Preparation of Green tea Extract

Dry leaves of *Thea sinensis* were collected from green tea farm at Boseong County, Cheollanamdo Province, South Korea. Powdered green tea leaves (100 g) were extracted at 100°C for one hour, and after cooling at 4°C for 12 hours the precipitate was removed by centrifugation at 5000×g for 30 min. Evaporation of the filtrate was made in the dryer and then grinded into powder. Finally, this powder was shaken with ether for 10 hours, and then after removing ether layer the supernatant was vaporized in the spray-dryer to give dried water-soluble fraction into powdered form (9.1 g). The working solution of this crude extract was prepared by dissolving in 0.9% NaCl solution on the day of each experiment, and filtered before administration.

Drugs and Their Sources

The following drugs were used: green tea extract (gift from professor Byung-Rai Lee, Department of Biochemistry, College of Medicine, Chosun University, Gwangju, Korea), acetylcholine chloride, norepinephrine bitartrate, and methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (BAY-K-8644) were purchased from Sigma Chemical Co., U.S.A. Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required except Bay-K-8644, which 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

Effects of CUMC6335 and Epigallocatechin Gallate (EGCG) on CA Secretion Evoked by ACh and High K^+ in the Perfused Rat Adrenal Gland

After the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 hr, basal CA release from the isolated perfused rat adrenal glands amounted to 20±2.1 ng/2 min (n=8). It has been shown that CUMC6335 in a dose-dependent fashion inhibited the contractile responses of the isolated rat aortic strip induced by phenylephrine or high potassium (Lim *et al.*, 2003). Therefore, it was decided initially to examine the effects of CUMC6335 on CA secretion from perfused rat adrenal glands evoked by cholinergic receptor stimulation as well as membrane depolarization. Secretagogues were given at 15 min-intervals, and CUMC6335 was introduced for 60 min after obtaining the control secretory response of each secretagogue. In the present study, it was found that CUMC6335 itself did not affect basal CA output (data not shown).

When ACh (5.32×10^{-3} M) in 0.05 mL volume was injected into the perfusion stream, the amount of CA secreted was 338 ± 36 ng for 4 min. However, in 8 adrenal glands, the pretreatment with CUMC6335 (100 μg/mL) for 60 min significantly inhibited ACh-stimulated CA secretion to ~28% of the control

response in a time-dependent manner (Fig. 1). However, in the presence of EGCG (8 μg/mL) for 60 min, ACh-stimulated CA secretion was not affected, as compared to the control release of CA (228±26 ng for 0-4 min) as shown in Fig. 2. Also, it has earlier been found that depolarizing agent such as KCl stimulates sharply CA secretion. In the present work, excess K^+ (5.6×10^{-2} M)-stimulated CA secretion in the presence of CUMC-6335 (100 μg/mL) was significantly inhibited to ~50% of the corresponding control secretion (165±8 ng for 0-4 min) from 8 glands (Fig. 3). However, it was not changed even in the presence of EGCG (8 μg/mL) for 60 min compared to the corre-

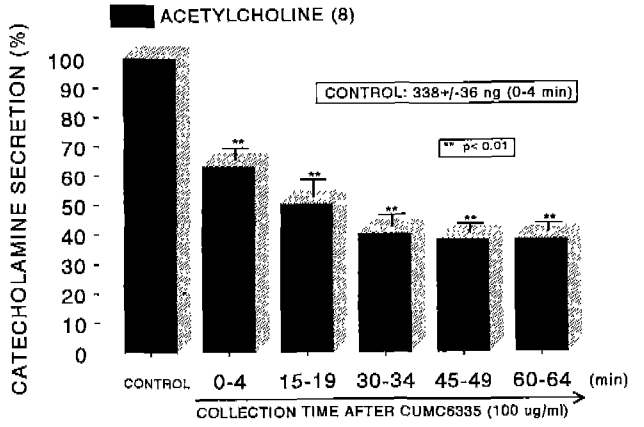

Fig. 1. Effects of CUMC6335 on the secretory responses of catecholamines (CA) evoked by acetylcholine from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) was induced "BEFORE" and "AFTER" preloading with CUMC6335 (100 μg/mL) for 60 min. Number in the parenthesis indicates the number of experimental rat adrenal glands. Vertical bars represent the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from adrenal gland (% of the control). Abscissa: collection time (min). Statistical difference was obtained by comparing the correspond-ing BEFORE (control) with each period "AFTER" the initiation of CUMC6335 perfusion. Perfusates were collected for 4 minutes at 15 min intervals. **: P<0.01.

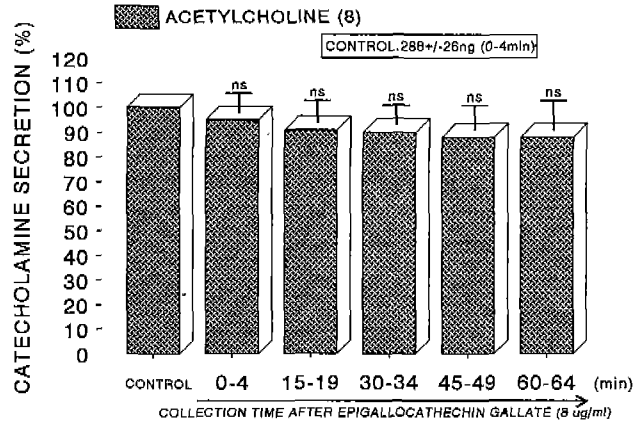

Fig. 2. Effects of epigallocatechin gallate on the secretory responses of catecholamines (CA) evoked by acetylcholine from the isolated perfused rat adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) was induced "BEFORE" and "AFTER" preloading with epigallocatechin gallate (8 μg/mL) for 60 min. Perfusates were collected for 4 minutes at 15 min intervals.Other legends are the same as in Fig. 1. There was no statistically differencebetween groups of control and after treatment with epigallocatechin gallate.

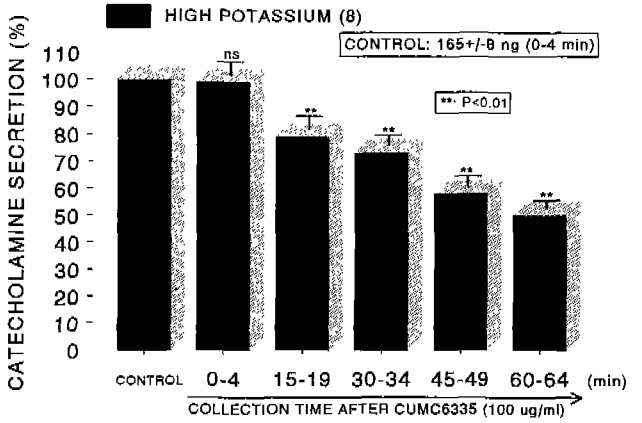

Fig. 3. Effects of CUMC6335 on the secretory responses of catecholamines (CA) evoked by high potassium from the isolated perfused rat adrenal glands. CA secretion by a single injection of high potassium (5.6×10^{-2} M) was induced "BEFORE" and "AFTER" preloading with CUMC6335 (100 μg/mL) for 60 min. Perfusates were collected for 4 minutes at 15 min intervals. Other legends are the same as in Fig. 1. **: P<0.01, ns: not statistically significant.

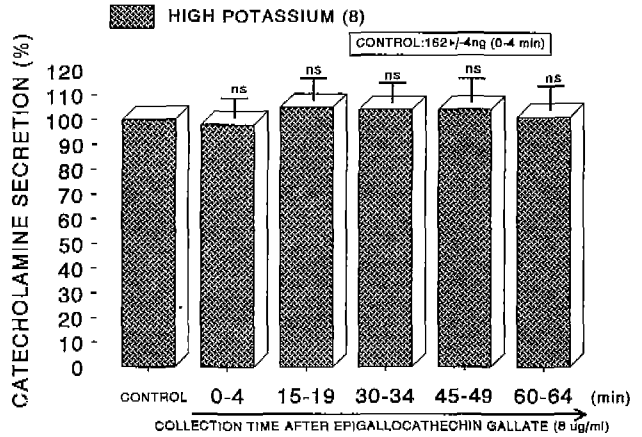

Fig. 4. Effects of epigallocatechin gallate on the secretory responses of catecholamines (CA) evoked by high potassium from the isolated perfused rat adrenal glands. CA secretion by a single injection of high potassium (5.6×10^{-2} M) was induced "BEFORE" and "AFTER" preloading with epigallocatechin gallate (8 μg/mL) for 60 min. Perfusates were collected for 4 minutes at 15 min intervals.Other legends are the same as in Fig. 1. There was no statistically differencebetween groups of control and after treatment with epigallocatechin gallate.

sponding release of CA (162±4 ng for 0-4 min) as depicted in Fig. 4.

Effects of CUMC6335 and Epigallocatechin Gallate (EGCG) on CA Secretion Evoked by Bay-K-8644 in the Perfused Rat Adrenal Glands

It has been found that Bay-K-8644 is a calcium channel activator that causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm *et al.*, 1982; Wada *et al.*, 1985a), and enhances basal Ca^{2+} uptake (Garcia *et al.*, 1984) and CA release (Lim *et al.*, 1992). Therefore, it was of interest to examine the effects of CUMC6335 on Bay-K-8644-evoked CA secretion from the isolated perfused rat adrenal glands. Fig. 5 shows the inhibitory effect of CUMC6335 on Bay-K-8644-evoked CA secretory responses. In the absence of CUMC6335, Bay-K-8644 (10^{-5} M) given into the perfusion stream evoked CA secretion of 112±10 ng (0-4 min) from 8 rat adrenal glands. However, in the presence of CUMC6335 (100 μg/mL), Bay-K-8644-stimulated CA secretion was inhibited to 0 ~78% of the corresponding control release. However, in the presence of EGCG (8 μg/mL) for 60 min, ACh-stimulated CA secretion was not affected, as compared to the control release of CA (120±15 ng for 0-4 min) as shown in Fig. 6.

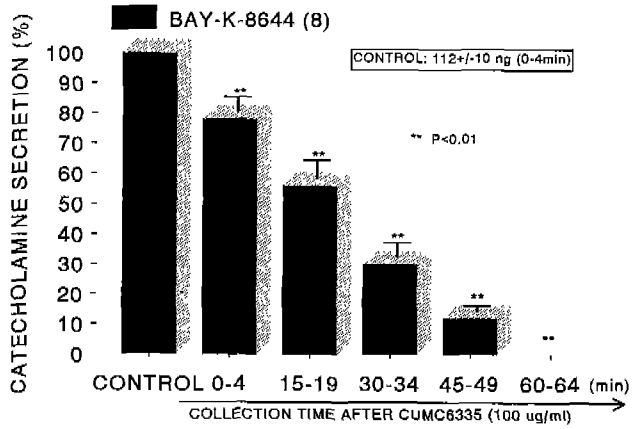

Fig. 5. Effects of CUMC6335 on the secretory responses of catecholamines (CA) evoked by Bay-k-8644 from the isolated perfused rat adrenal glands. CA secretion by a single injection of Bay-k-8644 (10^{-5} M) was induced "BEFORE" and "AFTER" preloading with CUMC6335 (100 μg/mL) for 60 min. Perfusates were collected for 4 minutes at 15 min intervals. Other legends are the same as in Fig. 1. **: P<0.01.

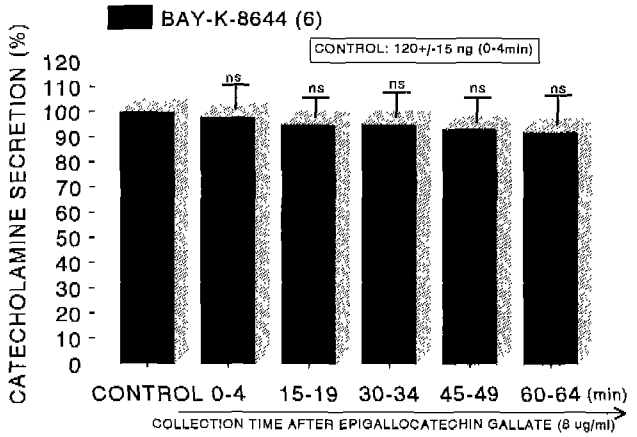

Fig. 6. Effects of epigallocatechin gallate on the secretory responses of catecholamines (CA) evoked by Bay-k-8644 from the isolated perfused rat adrenal glands. CA secretion by a single injection of Bay-k-8644 (10^{-5} M) was induced "BEFORE" and "AFTER" preloading with epigallocatechin gallate (8 μg/mL) for 60 min. Perfusates were collected for 4 minutes at 15 min intervals. Other legends are the same as in Fig. 1. ns: Statistically not significant.

DISCUSSION

The present experimental results have suggested that CUMC6335 inhibits greatly CA secretory responses evoked by ACh, high potassium and Bay-K-8644 from the isolated perfused rat adrenal gland, and that this inhibitory effect may be exert through the direct inhibition of calcium influx into the rat adrenomedullary chromaffin cells.

In support of this idea, tannins contained in green tea are found to induce the depressor effect in rat with renal hypertension (Yokozawa *et al.*, 1994). Extracts of tea (Fitzpatrick *et al.*, 1992) and flavonoids found in tea (Fitzpatrick *et al.*, 1993) have been shown to give vasodilator effects. In a cohort of Norwegian men and women, higher consumption of black tea was associated with lower SBP (Stensvold *et al.*, 1992). In terms of these findings, the results obtained from the present study seem likely that CUMC6335 can cause the depressor effect by the inhibition of CA secretion from the adrenal medulla. The present findings appeared to contribute at least partly to the facts that extracts of tea (Fitzpatrick *et al.*, 1992) and flavonoids found in tea (Fitzpatrick *et al.*, 1993) produced vasodilator effects, but not to the fact that tea ingestion in the normotensive men caused larger acute increases in blood pressure than caffeine alone (Hodgson *et al.*, 1999).

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. Based on this fact, the present findings that CUMC6335 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 CUMC6335 causes vasodilatation through Ca^{2+} antagonism in the isolated rat aorta (Lim *et al.*, 2003).

These experimental results indicate that CUMC6335-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 CUMC6335 may inhibit CA secretion evoked by nicotinic stimulation from the splanchnic nerve ending through the blockade of nicotinic receptors. The release 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 shown to be caused through stimulation of both nicotinic and muscarinic receptors in guinea-pig adrenal gland (Nakazato *et al*, 1988) as well as in the perfused rat adrenal glands (Lim & Hwang, 1991).

In the present study, CUMC6335 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 CUMC6335 may inhibit Ca^{2+} influx to the rat adrenomedullary 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 (Fisher, Holz & Agronoff, 1981; Yanagihara *et al*, 1979). It has been also known that the activation of nicotinic receptors stimulates CA secretion by increasing 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 (1985b) 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 CUMC6335 indicates strongly that this inhibitory effect of CUMC6335 is exerted through the direct inhibition of calcium influx into the rat adrenal chromaffin cells. Furthermore, slight elevation in the extracellular potassium concentration increases both the frequency 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.

However, in the present study, the pretreatment with EGCG failed to affect the secretion of CA evoked by ACh and high K^+ as well as by Bay-K-8644. EGCG is well known to be a major component of various catechins found in green tea. This finding suggests that CUMC6335-induced inhibitory action of the CA secretion is unlikely mediated at least by polyphenols found in green tea. Moreover, the result obtained from the present study is consistent with the previous finding that EGCG did not affect phenylephrine- as well as high potassium-induced contractile response of the isolated rat aorta. It supports that the inhibitory effect of CUMC6335 on CA secretion is not associated to the effects of catechins including EGCG contained in green tea.

In contrast, it has been shown that (–) epicatechin also concentration-dependently relaxed U46619-contracted arteries without the functional endothelium. It is unlikely that (–) epicatechin acts as an antagonist at prostaglandin receptors to cause relaxation since it reduced arterial contraction induced by other vasoconstrictors, such as phenylephrine and endothelin-1 (Huang *et al.*, 1998). The endothelium-independent relaxation induced by (–) epicatechin may be partly mediated through inhibition of Ca^{2+} influx through voltage-sensitive Ca^{2+} channels in vascular smooth muscle cells because (–) epicatechin

significantly reduced the high K^+-induced contraction in the same preparation (Huang *et al.*, 1998). Recently, it has been also found that (−) epicatechin could act on endothelium to increase intracellular Ca^{2+} and nitric oxide release, which may account for the endothelium-dependent relaxation (Huang *et al.*, 1999). In addition, (−) epicatechin-induced relaxation in endothelium-intact tissues may be also mediated by nitric oxide-dependent activation of iberiotoxin-sensitive K^+ channels. These mechanisms may be associated with a beneficial effect of green tea epicatechins on vascular system (Huang *et al.*, 1999). Recently, it has been shown that (−)-EGCG can facilitate the cholinergic ganglion transmission possibly by increasing the amount of ACh released and, together with its previously described depolarizing action on myenteric neurons, may modulate the activity of the myenteric plexus of the guinea-pig ileum (Katayama *et al.*, 2002). However, these (−) epicatechins effects are not agreement with the present result that EGCG failed to alter the CA secretory responses evoked by ACh and high potassium in the isolated perfused rat adrenal medulla. Moreover, these results was in agreement with the recent finding that EGCG did not affect the contractile responses induced by phenylephrine and high potassium in the isolated rat aortic strips (Lim *et al.*, 2003). Anyway, the effects of various catechins remain to be investigated in the future.

In conclusion, the present study using the isolated perfused rat adrenal glands suggested that CUMC6335 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 CUMC6335 components, through inhibition of CA secretion from adrenal medullary chromaffin cells and consequent reduction of the CA level in the circulation. It seems likely that there is much difference in mode of action between CUMC6335 and EGCG.

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