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Comparison of Green Tea Extract and Epigallocatechin Gallate on Secretion of Catecholamines from the Rabbit Adrenal Medulla#

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The present study was designed to examine the effects of green tea extract (CUMC6335) and epigallocatechin gallate (EGCG) on secretion of catecholamines (CA) in the isolated perfused rabbit adrenal gland. In the presence of CUMC6335 (200 μg/mL) into an adrenal vein for 60 min, CA secretory responses evoked by ACh (5.32 mM), high K^+ (56 mM), DMPP (100 μM for 2 min), and Bay-K-8644 (10 μM for 4 min) from the isolated perfused rabbit adrenal glands were greatly inhibited in a time-dependent fashion. However, EGCG (10 μg/mL) did not affect CA release evoked by ACh, high K^+, and Bay-K-8644. CUMC6335 itself failed to affect basal catecholamine output. Taken together, these results demonstrate that CUMC6335 inhibits CA secretion evoked by stimulation of cholinergic nicotinic receptors, as well as the direct membrane depolarization from the isolated perfused rabbit adrenal gland. It is thought that this inhibitory effect of CUMC6335 may be due at least in part to the blocking action of the L-type dihydropyridine calcium channels in the rabbit adrenomedullary 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.

Key words: Green tea extract (CUMC6335), Epigallocatechin gallate (EGCG), Catecholamine (CA) Secretion, Blockade of Nicotinic Receptors

INTRODUCTION

It is well-known that green tea, a 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, 1996). 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. 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 produce 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 clinically-measured blood pressure (Bingham *et al.*, 1997). Moreover, in older 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

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reduced arterial contraction induced by other vasoconstrictors, such as phenylephrine and endothelin-1 (Huang *et al.*, 1998). 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.

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 consumption (Hodgson *et al.*, 1999). Despite these results, there has been remarkably little research on the effects of tea ingestion on blood pressure. 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. Since catechin-polyphenols are known to be capable of inhibiting catechol-*O*-methyl-transferase (the enzyme that degrades NE), and caffeine to inhibit trancellular phosphodiesterases (enzymes that break down NE-induced cAMP), it is proposed that the green tea extract, *via* its catechin-polyphenols and caffeine, is effective in stimulating thermogenesis, by relieving inhibition at different control points along the NE-cAMP axis (Dulloo *et al.*, 2000). More recently, it has been found that green tea extract causes relaxation in the isolated aortic strips of the rat *via* the blockade of adrenergic α_1-receptors, in addition to the unknown direct mechanism (Lim *et al.*, 2003). It also inhibits CA secretion evoked by stimulation of cholinergic nicotinic receptors as well as the direct membrane depolarization from the isolated perfused rat adrenal gland (Lim *et al.*, 2003).

The present study was therefore designed to examine the effects of green tea extract (CUMC6335) on CA secretion from the isolated perfused model of the rabbit adrenal medulla, in comparison with the responses to epigallocatechin gallate (EGCG), one of the most powerful active catechin components derived from green tea, and to investigate whether there is a animal difference in the mode of action between rat and rabbit.

MATERIALS AND METHODS

Experimental procedure

White male New Zealand rabbits, weighing 2 to 3 kilograms, were anesthetized intravenously with thiopental sodium (40 mg/kg). The abdomen of the anesthetized rabbit 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. Water at 37 ± 1°C was continuously circulated through the chamber.

Perfusion of adrenal gland

The adrenal glands were perfused by means of a peristaltic pump (WIZ Co.) at a rate of 0.8 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 and DMPP (100 μM) for 2 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, but for the responses to DMPP it took 8 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 or EGCG on the spontaneous and evoked secretion, the adrenal gland was perfused with Krebs solution containing CUMC6335 or EGCG for 60 min immediately after the perfusate was collected for a short period of time (background sample). The medium was then changed to the one containing the stimulating agent, and the perfusates were collected for the same period of time as that for the background sample. Generally, the adrenal gland's perfusate was collected in chilled tubes.

Measurement of catecholamines

The 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 the control samples (unstimulated). The sample blanks were also the lowest for perfusates of both the stimulated and non-stimulated groups. 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 statistically significant, unless specifically noted in the text. Values given in the text refer to means and the 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 a 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 h the precipitate was removed by centrifugation at 5000 × g for 30 min. Evaporation of the filtrate was done in a dryer and then ground into powder. Finally, this powder was shaken together with ether for 10 h, and then after removing the 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: epigallocatechin gallate (EGCG), acetylcholine chloride, norepinephrine bitartrate, 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP), 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. The 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 EGCG on CA secretion evoked by ACh and high K^+ in a perfused rat adrenal gland

After the initial perfusion with oxygenated Krebs-bicarbonate solution for 1 h, 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 of interest to examine the effects of CUMC6335 on CA secretion from a perfused rabbit 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.

When ACh (5.32×10^{-3} M) in 0.05 mL volume was injected into the perfusion stream, the amount of CA secreted was 469 ± 87 ng for 4 min. Pretreatment in 8 adrenal glands, with CUMC6335 (200 μg/mL) for 60 min, significantly inhibited ACh-stimulated CA secretion by 27~64% of the control response, in a time-dependent manner (Fig. 1-lower). However, in the presence of EGCG (10 μg/mL) for 60 min, ACh-stimulated CA secretion was not affected, as compared to the control release of CA (304 ± 20 ng for 0-4 min) as shown in Fig. 1-lower. It had also been found that depolarizing agents, such as KCl, sharply stimulate CA secretion. In the present work, excess K^+ (5.6×10^{-2} M)-stimulated CA secretion in the presence of CUMC6335 (200 μg/mL) was significantly inhibited in a range of 0~59% of the corresponding control secretion (272 ± 46 ng for 0-4 min) from 8 glands (Fig. 2-upper). However, in the presence of EGCG (10 μg/mL) for 60 min, there was no change in excess K^+-evoked CA secretion in comparison with the corresponding release of

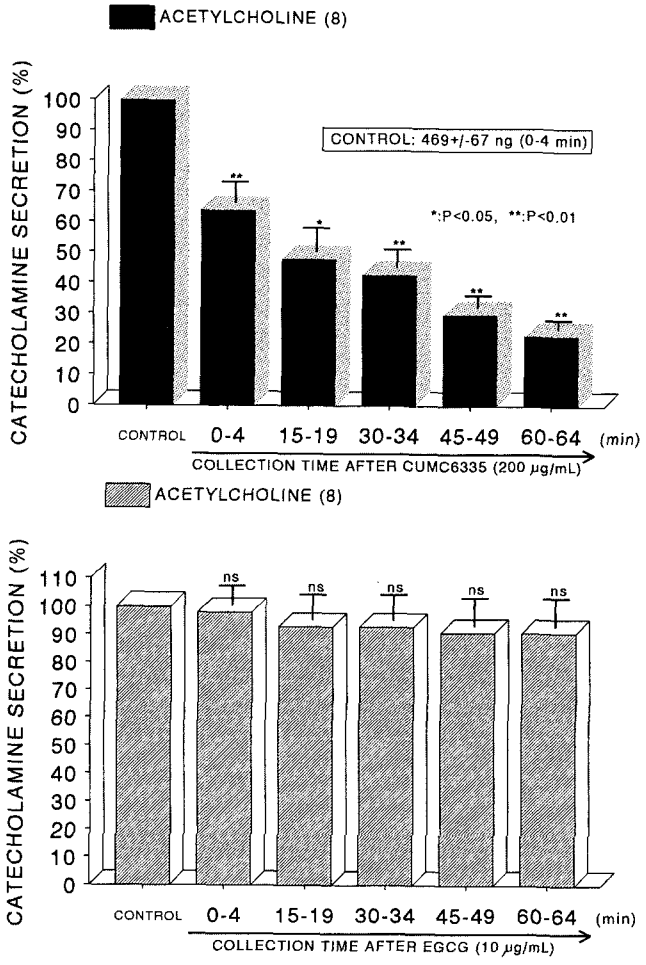

Fig. 1. Effects of CUMC6335 (green tea extract, **upper**) and epigallocatechin gallate (EGCG, **lower**) on the secretory responses of catecholamines (CA) evoked by acetylcholine from the isolated perfused rabbit adrenal glands. CA secretion by a single injection of ACh (5.32×10^{-3} M) was induced "BEFORE" and "AFTER" preloading with CUMC6335 (200 μg/mL) or EGCG (10 μg/mL) for 60 min. Number in the parenthesis indicates the number of experimental rabbit 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 corresponding "BEFORE" (control) with each period "AFTER" the initiation of CUMC6335 perfusion. Perfusates were collected for 4 min at 15 min intervals. There was no statistically difference between groups before and after treatment with EGCG. ns: Statistically not significant.

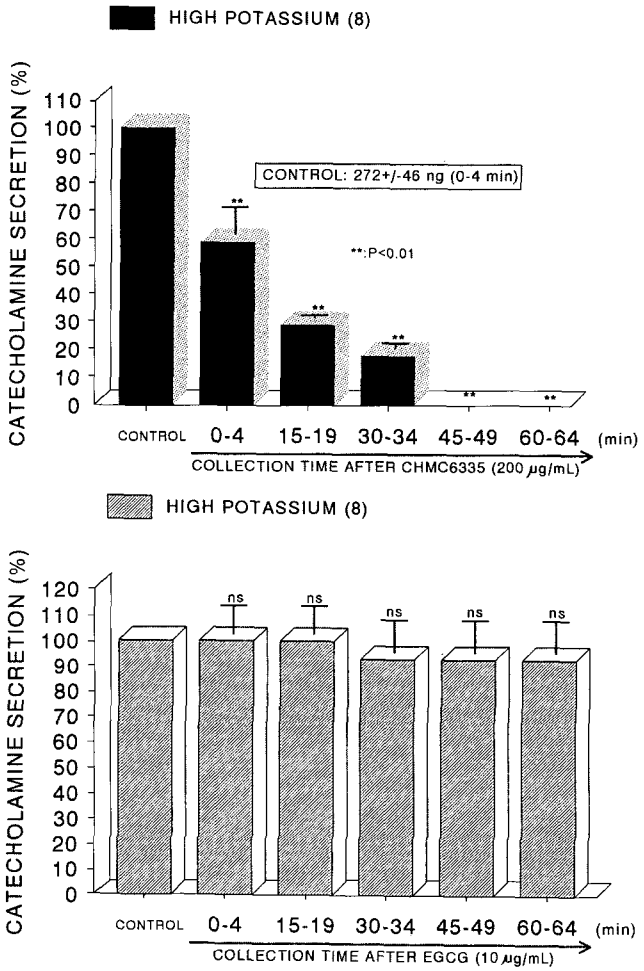

Fig. 2. Effects of CUMC6335 (green tea extract, **upper**) and epigallocatechin gallate (EGCG, **lower**) on the secretory responses of catecholamines (CA) evoked by high potassium from the isolated perfused rabbit adrenal glands. CA secretion by a single injection of high potassium (5.6×10^{-2} M) was induced "BEFORE (control)" and "AFTER" preloading with CUMC6335 (200 μg/mL) or EGCG (10 μg/mL) for 60 min. Perfusates were collected for 4 min at 15 min intervals. Other legends are the same as in Fig. 1 There was no statistically difference between groups before and after treatment with EGCG. ns: Statistically not significant.

CA (162 ± 4 ng for 0-4 min) as depicted in Fig. 2-lower.

Effects of CUMC6335 and EGCG on CA secretion evoked by Bay-K-8644 in the perfused rat adrenal glands

Bay-K-8644 has been found to be a calcium channel activator that causes positive inotropy and vasoconstriction in isolated tissues and intact animals (Schramm *et al.*, 1983; Wada *et al.*, 1985a). It also 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. 3-upper 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 213 ± 27 ng (0-4 min) from 6 rat adrenal glands. However, in the presence of CUMC6335 (200 μg/mL), Bay-K-8644-stimulated CA secretion was inhibited from 28 to 70% of the corresponding control release. However, in the presence of EGCG (10 μg/mL) for 60 min, Bay-K-8644-stimulated CA

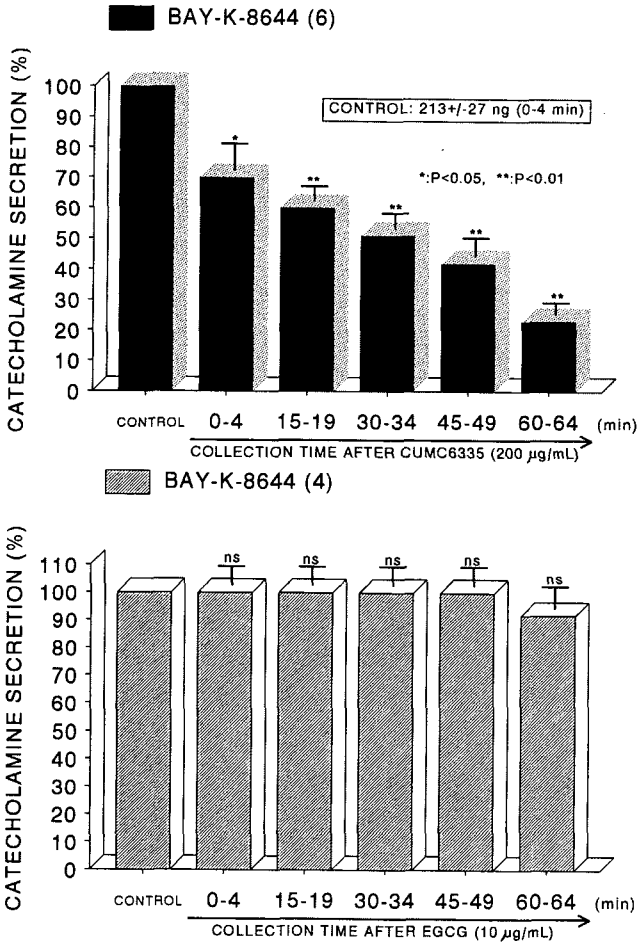

Fig. 3. Effects of CUMC6335 (green tea extract, **upper**) and epigallocatechin gallate (EGCG, **lower**) on the secretory responses of catecholamines (CA) evoked by Bay-K-8644 from the isolated perfused rabbit adrenal glands. CA secretion by a single injection of Bay-K-8644 (10^{-5} M) was induced "BEFORE" and "AFTER" preloading with CUMC6335 (200 μg/mL) or EGCG (10 μg/mL) for 60 min. Perfusates were collected for 4 min at 15 min intervals. Other legends are the same as in Fig. 1. There was no statistically difference between groups before and after treatment with EGCG. ns: Statistically not significant.

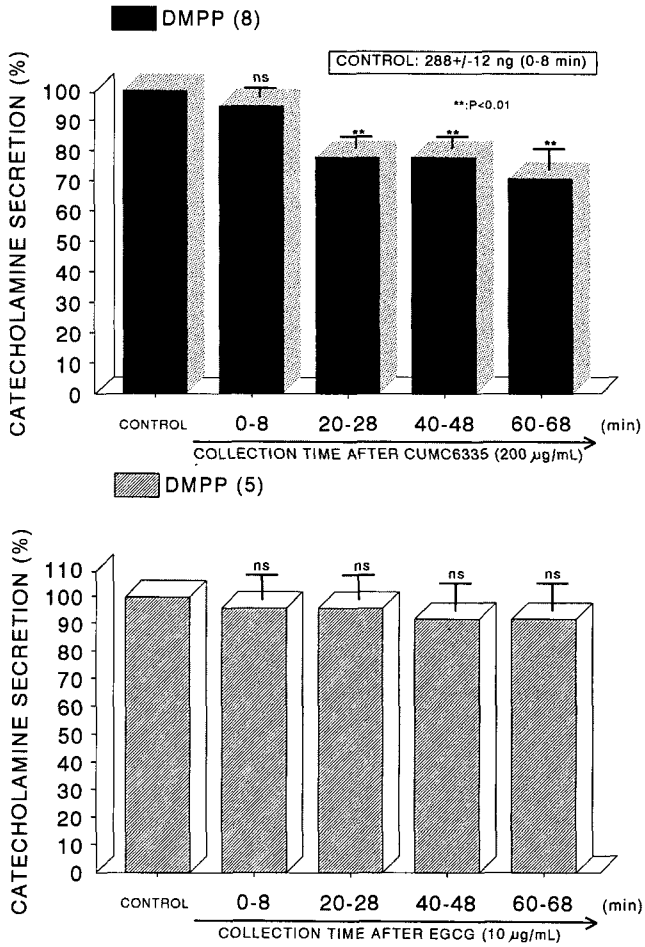

Fig. 4. Effects of CUMC6335 (green tea extract, **upper**) and epigallocatechin gallate (EGCG, **lower**) on the secretory responses of catecholamines (CA) evoked by DMPP from the isolated perfused rabbit adrenal glands. CA secretion by the perfusion of DMPP (10^{-4} M) for 2 min was induced "BEFORE" and "AFTER" preloading with CUMC6335 (200 μg/mL) or EGCG (10 μg/mL) for 60 min. Perfusates were collected for 8 min at 20 min intervals. Other legends are the same as in Fig. 1. There was no statistically difference between groups before and after treatment with EGCG. ns: Statistically not significant.

secretion was not affected, in comparison to the control release of CA (144 ± 10 ng for 0-4 min) as shown in Fig. 3-lower.

Effects of CUMC6335 and EGCG on DMPP-evoked CA secretion in the perfused rat adrenal gland

As shown in Fig. 1~3, it is suggested that CUMC6335 possesses the antagonist effect of neuronal cholinergic nicotinic receptors in the rabbit adrenal medulla. Therefore, we tried to examine the effects of CUMC6335 and EGCG on DMPP-evoked CA releasing response in the perfused rabbit adrenal gland. DMPP (10^{-4} M), a selective nicotinic receptor agonist in autonomic sympathetic ganglia, when perfused through the rabbit adrenal gland, evoked a sharp and rapid increase in CA secretion. As shown in Fig. 4-upper, DMPP (10^{-4} M)-stimulated CA secretion following the loading with CUMC6335 (200 μM) was depressed by 71% of the corresponding control secretion (288 ± 12 ng/0-8 min). However, in the presence of EGCG (10 μg/mL) for 60 min, DMPP (10^{-4} M)-evoked CA secretory response was not changed in comparison with the corresponding release of CA (301 ± 34 ng for 0-8 min) as shown in Fig. 4-lower.

DISCUSSION

The present results have demonstrated that CUMC6335 greatly inhibits CA secretory responses evoked by ACh, high potassium, DMPP and Bay-K-8644 from the isolated perfused rabbit adrenal gland, and that this inhibitory effect may be exert at least partially through the direct

inhibition of L-type dihydropyridine calcium channels in the rabbit adrenomedullary chromaffin cells. It seems likely that there is no difference in mode of action between rabbits and rats.

In support of this idea, similarly, we have found that green tea extract causes the relaxation in the isolated aortic strips of the rat *via* the blockade of adrenergic α_1-receptors, in addition to the unknown direct mechanism (Lim *et al.*, 2003). It inhibits CA secretion evoked by stimulation of cholinergic nicotinic receptors as well as by the direct membrane depolarization from the isolated perfused rat adrenal gland (Lim *et al.*, 2003). Furthermore, 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 a depressor effect, at least partly, 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 hormones. Upon excitation of the splanchnic nerves, ACh is released from the nerve terminals, and then 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 rabbit adrenal medulla seem to support the fact that CUMC6335 causes vasodilatation through Ca^{2+} antagonism in the isolated rat aorta (Lim *et al.*, 2003), and also reduced the CA release evoked by nicotinic receptor stimulation *via* the inhibition of Ca^{2+} channel in rat adrenomedullary chromaffin cells (Lim *et al.*, 2003).

These experimental results indicate that the inhibition of CUMC6335 on CA secretion induced 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 β-hydroxylase through a calcium dependent secretory process (Dixon *et al.*, 1975; Viveros *et al.*, 1968). In 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 blockage 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 occurring in noradrenergic axons (Douglas, 1968; Sorimachi and 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 and 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 rabbit 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, and 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 and Wakade, 1983; Lim and Hwang, 1991) and isolated bovine adrenal chromaffin cells (Kilpatrick *et al.*, 1981, 1982; Knight and 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 activation of voltage-dependent Ca^{2+} channels by altering the membrane potentials, whereas high K^+ directly activates voltage-dependent Ca^{2+} channels without increasing Na^+ influx. The finding in the present study indicate that high potassium-induced CA secretory response was markedly depressed by pretreatment with CUMC6335, which strongly indicates that this inhibitory effect of CUMC6335

is exerted through the direct inhibition of calcium influx into the rabbit 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 and 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, DMPP, 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. Furthermore, we found very similar results in the perfused rat adrenal medulla (Lim *et al.*, 2003). These results support that the inhibitory effect of CUMC6335 on CA secretion is not associated with the effects of catechins including EGCG contained in green tea.

In contrast, it has been shown that (-) epicatechin, in a concentration-dependent manner, 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). 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 (-) epicatechin's effects are not in agreement with the present results that EGCG failed to alter the CA secretory responses evoked by ACh, DMPP, and high potassium in the isolated perfused rabbit adrenal medulla. Moreover, these results were in agreement with the recent finding that EGCG did not affect both the contractile responses induced by phenylephrine and high potassium in the isolated rat aortic strips (Lim *et al.*, 2003), and also the CA secretory responses evoked by chlolinergic nicotinic stimulation (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 rabbit 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 rabbit adrenal medullary chromaffin cells and consequent reduction of the CA level in the circulation. It seems likely that there is a big difference in mode of action between CUMC6335 and EGCG while no difference between rabbit and rat.

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