Natural Product Sciences
10(5) : 228-236 (2004)

Green Tea Extract (CUMC6335), not Epigallocatechin Gallate, Causes Vascular Relaxation in Rabbits

[1]Dong Yoon Lim*, Young Joo Baek[1], and Eun Bang Lee[2]

[1]*Department of Pharmacology, College of Medicine, Chosun University, Gwangju 501-759, Korea*
[2]*Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea*

Abstract – The aim of the present study was to examine whether green tea extract (CUMC6335) affects the blood pressure and the isolated aortic contractility of the rabbit in comparison with one of the most powerful active catechins, epigallocatechin gallate (EGCG). The phenylephrine (1-10 μM)-induced contractile responses were greatly inhibited in the presence of CUMC6335 (0.3-1.2 mg/ml). Also, high potassium (56 mM)-induced contractile responses were depressed in high concentration (0.6-1.2 mg/ml), but not affected in low concentration CUMC6335 (0.3 mg/ml). However, epigallocatechin gallate (EGCG, 4-12 μg/ml) did not affect the contractile responses evoked by phenylephrine and high K^+. The infusion of CUMC6335 with a rate of 20 mg/kg/30 min made a significant reduction in pressor responses induced by intravenous norepinephrine. However, EGCG (1 mg/kg/30 min) did not affect them. Collectively, these results obtained from the present study suggest that intravenous CUMC6335 causes depressor action in the anesthetized rat at least partly through the blockade of adrenergic α_1-receptors. CUMC6335 also causes the relaxation in the isolated aortic strips of the rabbit partly via the blockade of adrenergic α_1-receptors, in addition to the unknown direct mechanism. It seems that there is no species difference in the vascular effect between the rat and the rabbit.

Keywords – Green tea extract (CUMC6335), Epigallocatechin gallate (EGCG), vasorelaxation, adrenergic α_1-receptors blockade

Introduction

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). 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). Ingestion of caffeine results in a transient increase in blood pressure in subjects who have avoided caffeine for 12 hours 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). It has also 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).

*Author for correspondence
Fax: +82-62-227-4693; E-mail: dylim@chosun.ac.kr

In contrast to these results, extracts of tea (Fitzpatrick *et al.*, 1995) and flavonoids found in tea (Fitzpatrick *et al.*, 1993) have been shown to give vasodilator effects *in vitro*. Decaffeinated green tea could lower pressure and heart rate in mice (Henry and Stephens-Larson, 1984). 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). 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. It was shown that (–) epicatechin also reduced arterial contraction 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. Recently, it has been found that green tea extract causes the relaxation in the isolated aortic strips of the rat (Lim *et al.*, 2003), and also

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).

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). Despite these results, there seems to be controversy about the effects of green tea on blood pressure. Therefore, the present study was designed to examine the effects of green tea extract (CUMC6335) blood pressure in the anesthetized rabbit and contractile responses of isolated aortic strips of the rabbit, and to establish the mechanism of action, 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 used in the experiment. The animals were housed individually in separate cages, and food (Cheil Animal Chow) and tap water were allowed ad *libitum* for at least a week to adapt to experimental circumstances. On the day of experiment, a rabbit was anesthetized with intravenously thiopental sodium (40 mg/kg), and tied in supine position on fixing panel.

Isolation of aortic strips – The thorax was opened by a midline incision, and placing three hook retractors exposed the heart and surrounding area. The heart and portion of the lung were not removed, but pushed over to the right side and covered by saline-soaked gauge pads in order to obtain enough working space for isolating aortic vessel. The aorta was isolated from the proximal part of the heart to the vicinity of liver and immediately immersed in cold Krebs solution. The blood within the aorta was rapidly removed. The aorta was cut into the ring of 4-5 mm length.

Preparation of arterial cannulation – The animal was tied in supine position on fixing panel to insert a T-formed cannula into the trachea for securing free air passage. The rectal temperature was maintained at 37-38°C by a thermostatically controlling blanket and heating lamp throughout the course of the experiment.

Recording of mechanical activity – The ring segment of aorta was mounted in a muscle bath by sliding the ring over two parallel stainless-steel hooks (0.15 mm in diameter). The lower hook was fixed on bottom of the bath and the upper was connected to isometric transducer (Grass FT. 03). The signal from the transducer was displayed on a polygraph (Grass Instruments Model 79). The volume of bath was 25 ml and the bath solution was saturated with 95% O_2 and 5% CO_2 at 37°C. The composition (mM) of Krebs was: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MaCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The final pH of the solution was maintained at 7.4-7.5. During equilibration period of 2 hours, the resting tension was adjusted to 0.5 g. After the equilibration period, the ring was challenged with 35 mM KCl two times, and if it responded with contraction, the proper experiments were started. Vasoconstrictors were administered into the bath in order to obtain dose-response curves. In the subsequent experiments, under the presence of green tea extract, some vasoconstrictors were administered, respectively. The data were expressed as % of the control tension.

Measurement of blood pressure – In order to observe the change of arterial pressure, one of the femoral arteries was catheterized with polyethylene tubing [outside diameter (o.d.): 0.5 mm]. The tubing was connected to a pressure transducer (Gould Co., U.S.A.) and pulse of mean arterial blood pressure was recorded on a biological polygraph (Grass Co., U.S.A.) continuously. The chart speed was adjusted to 2 cm per minute. The artery tubing was filled with heparin solution (400 I.U) to prevent the blood coagulation during the experiment. Another cannulation with polyethylene tubing (o.d.: 0.3 mm) was made into a femoral vein for the administration of drugs and supplemental anesthetic agents as needed to maintain light surgical anesthesia. Each rabbit was left undisturbed for at least 30 minutes after completion of the operative procedures to permit cardiovascular parameters to be stabilized and drugs under investigation were administered at intervals of 60 minutes.

Statistical analysis – The statistical significance between groups was determined by the Student's *t*- and *ANOVA*-tests. 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 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 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: phenylephrine hydrochloride, potassium chloride (KCl), epigallocatechin 3-gallate and norepinephrine bitartrate (Sigma Chemical Co., U.S.A), thiopental sodium and heparin sodium (Daehan Choongwae Pharm. Co., Korea). Drugs were dissolved in distilled water (stock) and added to the normal Krebs or saline solution as required. Concentrations of all drugs used are expressed in terms of molar base and g.

Results

Effects of green tea extract (CUMC6335) on contractile responses induced by Phenylephrine (PE) and high K^+ in the rabbit aortic strips – The resting tension from the isolated rabbit aortic strips reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 120 min before the experimental protocol is initiated. The resting tension was adjusted to 0.5 g. The effects of CUMC6335 on PE- as well as high K^+-mediated contractile responses in the rabbit aorta were examined. In the present study, CUMC6335 itself did not produce any effect on the resting tension in the aortic strips isolated from the rabbit (data not shown). When 10^{-6} M and 10^{-5} M of PE concentrations were administered into the aortic bath, their active tensions amounted to 2.5 ± 0.2 g and 3.6 ± 0.3 g from the resting tension level, respectively. However, in the presence of CUMC6335 at 0.3, 0.6 and 1.2 mg/ml, respectively, 10^{-6} M PE-induced tensions were dose-dependently inhibited to 75-39% of the control contractile responses, respectively (Fig. 1). Moreover, 10^{-5} M PE-induced contractile responses were also dose-dependently inhibited to 88-57% of the control responses, respectively (Fig. 1).

High K^+ exerts two distinct effects on cells: (1) depolarization of cell membrane, and (2) depolarization-induced influx of calcium via voltage-dependent calcium channels (Wada *et al.*, 1985). When added through the bath, high potassium at the concentrations of 5.6×10^{-2} M, which is a membrane-depolarizing concentration, caused an increase in aortic contraction. As shown in Fig. 2, high

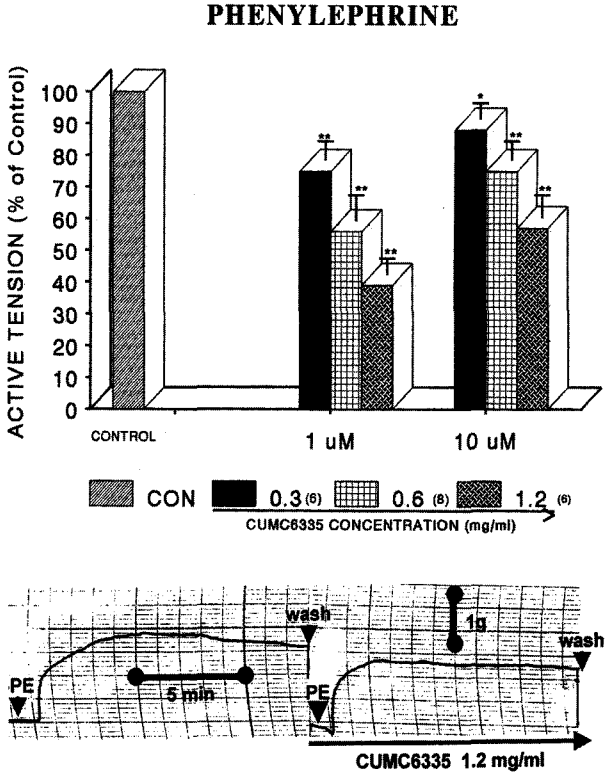

Fig. 1. Upper: Influence of green tea extract (CUMC6335) on phenylephrine (PE)-induced contractile responses in the isolated rabbit aortic strips. The contractile response was induced by adding 1 μM and 10 μM of PE, respectively after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. "CONTROL" and "AFTER" denote active tension induced evoked by PE before (CON) and after adding 0.3, 0.6 and 1.2 mg/ml of CUMC6335, respectively. Numeral in the parenthesis indicates number of experimental rabbit aortic strips. Vertical bars represent the standard error of the mean (S.E.M). Ordinate: the active tension (% of control). Abscissa: concentrations of PE (μM). Statistical difference was obtained by comparing the control with the CUMC6335-pretreated group. *: $P < 0.05$, **: $P < 0.01$. **Lower:** The typical tracing showing the effect of CUMC6335 on PE-induced contractile responses in the rabbit aortic strips. Left: PE-induced contractile response. Right: PE-induced contractile response in the presence of 1.2 mg/ml of CUMC6335. At arrow mark, the indicated dose (10^{-5} M) of PE was added to the bath. The chart speed was 5 mm/min.

potassium (5.6×10^{-2} M)-induced contractile response after pre-loading with 0.3 mg/ml of CUMC6335 did not show any significant difference as compared with the corresponding control response (2.8 ± 0.1 g). However, in the presence of CUMC6335 at concentrations of 0.6 and 1.2 mg/ml, high potassium (5.6×10^{-2} M)-induced contractile responses were 78-62% of their corresponding control responses in a dose-dependent fashion, respectively (Fig. 2). These results seem to be similar to the mode of action as shown in rat aortic strips (Lim *et al.*, 2003).

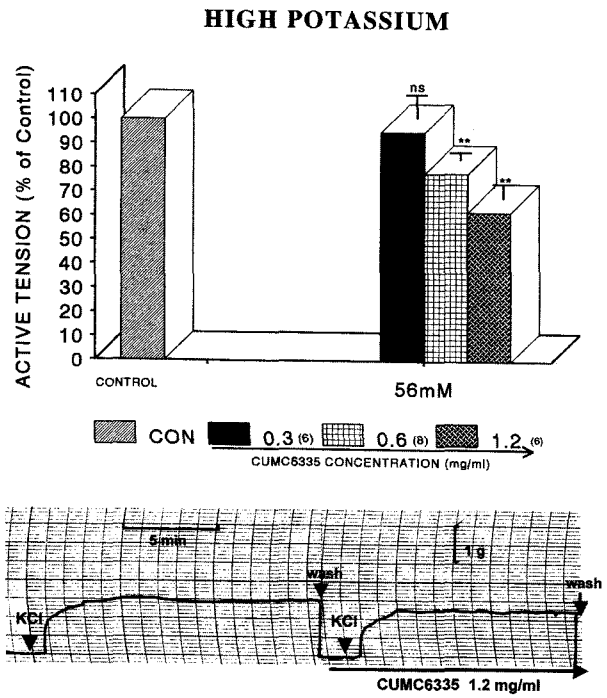

Fig. 2. Upper: Influence of green tea extract (CUMC6335) on high potassium-induced contractile responses in the isolated rabbit aorta. High potassium (56 mM) was added into the bath before and after pretreatment with 0.3, 0.6 1.2 mg/ml of CUMC6335, respectively. Other legends are the same as in Fig. 1. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant. **Lower:** The typical tracing showing the effect of CUMC6335 on high potassium (KCl)-induced contractile responses in the rabbit aortic strips. Left: KCl-induced contractile response. Right: KCl-induced contractile response in the presence of 1.2 mg/ml of CUMC6335. At arrow mark, the indicated dose (56 mM) of KCl was added to the bath. The chart speed was 5 mm/min.

Effects of epigallocatechin 3-gallate (EGCG) on contractile responses induced by PE and high K^+ in the rabbit aortic strips – Since EGCG is found to be a main constituent of green tea leaves, it was likely interesting to compare the effects of EGCG on the contractile responses induced by high potassium and PE. In the presence of 15 μg/ml of EGCG, the aortic contractile response evoked by PE (10^{-6} M) was 94% of the control in comparison with the corresponding control response (2.9 ± 0.4 g) from the resting tension level as depicted in Fig. 3. High potassium-induced contractile response before treatment with EGCG was 2.5 ± 0.4 g, while after pretreatment with 15 μg/ml of EGCG it amounted to 104% of the corresponding control response, which there was no statistically difference between control and EGCG-treated groups (Fig. 3).

Effects of green tea extract (CUMC6335) and epigallocatechin-3-gallate (EGCG) on norepinephrine-induced hypertensive responses in the anesthetized rabbit – Since CUMC6335 greatly inhibited PE-induced contractile responses of the isolated aortic smooth muscle as shown in Fig. 1, it is of interest to examine the effect of intravenous CUMC6335 on norepinephrine-evoked pressor responses. When cardiovascular parameters were stabilized for 30 min before the experimental protocols were initiated, the administration of physiological saline solution in a volume of 0.2 ml into a femoral vein did not cause any changes in both arterial blood pressure. In 7 rabbits, norepinephrine at doses of 1,0, 3.0 and 10.0 μg/kg caused dose-dependent pressor responses of 8 ± 1 mmHg, 18 ± 1 mmHg and 34 ± 4 mmHg from the original baseline (121 ± 8 mmHg), respectively. However, after infusion of CUMC6335 with a rate of 20 mg/kg/30min, they were significantly depressed to 5 ± 1 mmHg ($P < 0.05$), 10 ± 2

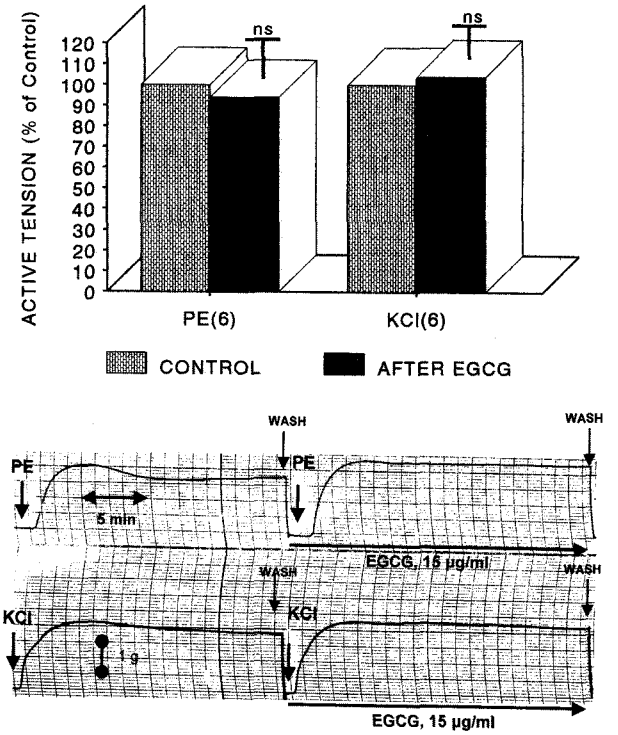

Fig. 3. Upper: Influence of epigallocatechin 3-gallate (EGCG) on phenylephrine (PE)- and high potassium (KCl)-induced contractile responses in the isolated rabbit aortic strips. The contractile response was induced by adding 10 μM of PE or 56 mM of KCl before (CONTROL) and after adding 15 μg/ml of EGCG. Other legends are the same as in Fig. 1. ns: Statistically not significant. Lower: The typical tracings showing the effect of epigallocatechin 3-gallate (EGCG) on phenylephrine (PE)- and high potassium (KCl)-induced contractile responses in the rabbit aortic strips. Upper panel: Left: PE-induced contractile response. Right: PE-induced contractile response in the presence of 15 μg/ml of EGCG. At arrow mark, the indicated dose (10^{-6} M) of phenylephrine was added to the bath. Lower panel: Left: KCl-induced contractile response. Right: KCl-induced contractile response in the presence of 15 μg/ml of EGCG. At arrow mark, the indicated dose (56 mM) of KCl was added to the bath. The chart speed was 5 mm/min. Other legends are the same as in Fig. 1.

mmHg ($P < 0.05$) and 22 ± 3 mmHg ($P < 0.05$) at the above same doses, respectively (Fig. 4). Fig. 6-lower shows that norepinephrine-evoked pressor responses are greatly attenuated after pretreatment with intravenous CUMC6335.

In order to compare to CUMC6335 effects, it was tried to examine the effect of EGCG on norepinephrine-evoked pressor responses. In 8 rabbits, norepinephrine at doses of 1,0, 3.0 and 10.0 μg/kg before the pretreatment with EGCG caused dose-dependent pressor responses of 10 ± 0.5 mmHg, 17 ± 1 mmHg and 32 ± 3 mmHg from the original baseline, respectively. However, after infusion of EGCG with a rate of 1 mg/kg/30 min, they were 11 ± 0.8 mmHg (ns), 18 ± 1 mmHg (ns) and 32 ± 3 mmHg (ns) at all the above same doses, respectively (Fig. 5). Fig. 5-lower shows that norepinephrine-evoked pressor responses are not affected after pretreatment with intravenous EGCG.

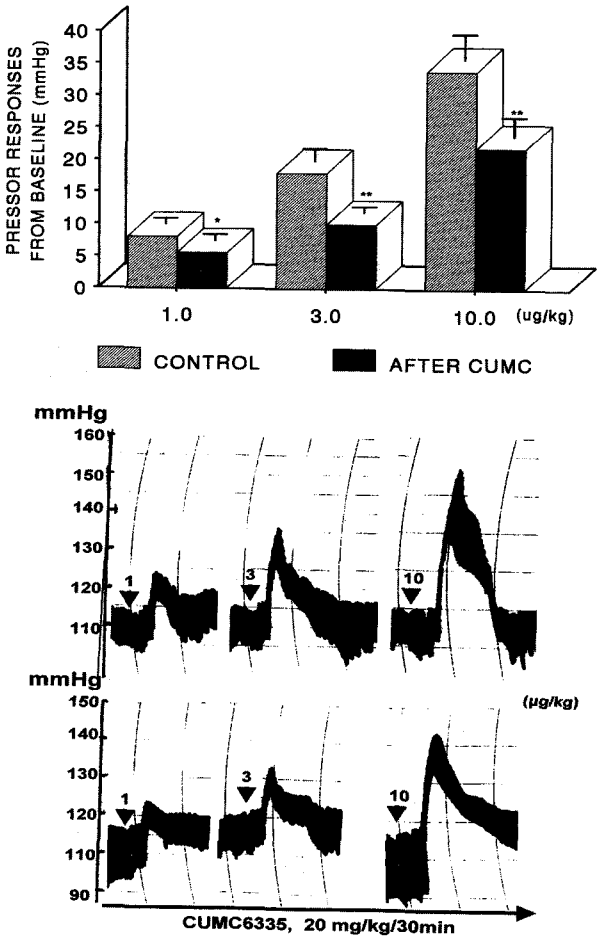

Fig. 4. Upper: Influence of green tea extract (CUMC6335) on intravenous norepinephrine (NE)-induced pressor response in anesthetized rabbits. Ordinate: Changes of blood pressure from baseline level in mmHg from 7 rabbits. Abscissa: Intravenous doses of NE in μg/kg. Vertical bar on top of each column indicates standard error of mean (S.E.M.). Statistical difference was obtained by comparing the control with the CUMC6335-pretreated group. The original base-line of arterial blood pressure was 121 ± 8 mmHg. *: $P < 0.05$, **: $P < 0.01$. **Lower:** The representative tracing of CUMC6335's effect on intravenous NE-induced pressor response in the anesthetized rabbit. At arrow marks, the indicated doses (1.0, 3.0 and 10.0 μgkg) of NE were administered into a femoral vein. Upper panel: NE only-induced hypertensive responses in a non-treated rabbit. Lower panel: NE-induced hypertensive responses in the CUMC6335-pretreated rabbit. CUMC6335 was infused into a femoral vein with a rabbit of 20 mg/kg/30 min. The chart speed was 20 mm/min.

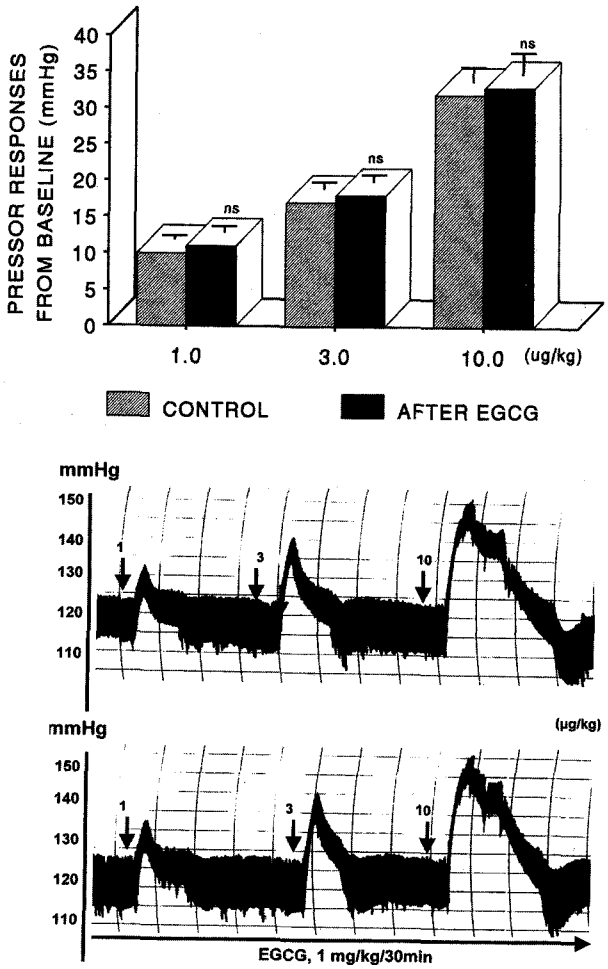

Fig. 5. Upper: Influence of epigallocatechin 3-gallate (EGCG) on norepinephrine (NE)-evoked pressor responses. EGCG was infused into a femoral vein with a rabbit of 1 mg/kg/30 min after obtaining the corresponding control responses of intravenous NE. Other legends are the same as in Fig. 5. ns: Statistically not significant. **Lower:** the representative tracing of EGCG effect on intravenous NE-induced pressor response in the anesthetized rabbit. At arrow marks, the indicated doses (1.0, 3.0 and 10.0 μgkg) of NE were administered into a femoral vein. Upper panel: NE only-induced hypertensive responses in a non-treated rabbit. Lower panel: NE-induced hypertensive responses in the EGCG-pretreated rabbit. EGCG was infused into a femoral vein with a rate of 1 mg/kg/30 min. The chart speed was 20 mm/min.

Discussion

The present experimental results suggest that intravenous CUMC6335 causes the depressor action in the anesthetized rabbit at least partly through the blockade of adrenergic α_1-receptors. It seems that CUMC6335 also causes vascular relaxation in the isolated aortic strips of the rabbit via the blockade of adrenergic α_1-receptors, in addition to the unknown mechanism. However, EGCG did not. This indicates that there is no species difference in the vascular effect between the rat and the ratbbit.

In support of these data, the present results are similar to the mode of action as shown in the isolated rat aortic strips (Lim *et al.*, 2003). Yokogoshi and his coworkers (1995) have shown that high doses (20 mg/kg) of theanine decreased significantly the blood pressure in spontaneously hypertensive rats, while the same doses to Wistar Kyoto rats did not alter it. Theanine is a novel amino acid found only in tea (Ballentine *et al.*, 1997). Moreover, it has been also reported that GABA-rich tea seems not only to decrease the established high blood pressure but also to prevent the development of hypertension in Dahl S rats fed a high salt diet (Abe *et al.*, 1995). 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.*, 1995) and flavonoids found in tea (Fitzpatrick *et al.*, 1993) have been shown to give vasodilator effects *in vitro*. In terms of these findings, the results obtained from the present study seem likely that CUMC6335 can cause the depressor effect.

In general, among drugs which interfere with peripheral sympathetic function, adrenergic α-receptor blocking agents alone cause reversal of the epinephrine pressor response (Constantine *et al.*, 1973). When epinephrine is administered to untreated animals, its α-agonist properties predominate, resulting in a rise in mean arterial pressure. However, in the presence of adrenergic α-receptor blockade, the peripheral β_2-agonist properties of epine-phrine predominate and a fall in arterial pressure or reversal of the pressor response is observed. In contrast, the pressor responses to norepinephrine are impaired by adrenergic α-receptor blockade, but are not reversed (Freis *et al.*, 1951) as this agent processes little β_2-agonist activity (Ablad *et al.*, 1975). In terms of the fact that PE-evoked contractile response is greatly depressed by CUMC6335, it is thought that CUMC6335 has vascular dilatatory activity at least partly through the adrenergic α-receptor blockade. In view of these reports, in the present work, the finding that CUMC6335 attenuated the norepinephrine-induced pressor responses demonstrates that CUMC6335 possesses the antagonistic activity of adrenergic α_1-receptors. However, It has been suggested that antioxidant (Rice-Evans *et al.*, 1995) and vasodilatory (Fitzpatrick *et al.*, 1993, 1995) polyphenolics in tea can attenuate the transient pressor effect of caffeine, and lower blood pressure during regular consumption. Huang and his colleagues (1999) have found that (−) epicatechin causes endothelium-dependent relaxation primarily mediated by NO and partially through NO-dependent activation of iberiotoxin-sensitive K^+ channels in rat isolated mesenteric arteries. However, the present study, the pretreatment with EGCG failed to affect the hypertensive responses evoked by intravenous nore-pinephrine. EGCG is well known to be a major component of catechins found in green tea. This finding suggests that CUMC6335-induced depressor effect is unlikely mediated by polyphenols found in green tea. Moreover, the result obtained from the present study that EGCG, a major component of various catechins did not affect PE- as well as high K^+-induced contractile response support that CUMC6335's vasorelaxation is not associated to the effects of catechins including EGCG contained in CUMC6335. Several previous studies have shown that green tea and black tea contain antioxidative polyphenols (Graham, 1992); polyphenols in green tea consist of flavon 3-ols such as (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epigallocatechingallate. One of the catechins, (−)-eigallocatechingallate (a main constituent of green tea leaves) significantly inhibited the promotion of tumors and carcinogenesis in animal experiments (Wang et al., 1989; Yoshizawa *et al.*, 1987; Conney *et al.*, 1992; Fujiki *et al.*, 1992; Wang *et al.*, 1992). Moreover, animal experiments have also shown the protective effects of green tea against cardiovascular diseases (Young *et al.*, 1967; Sano *et al.*, 1986; De Whalley *et al.*, 1990).

Generally, it well known that high K^+ opens voltage-dependent calcium channels by depolarizing the cell membrane of vascular smooth muscle, resulting in increased influx of extracellualr Ca^{2+} (Bolton, 1979; Schwartz and Taira, 1983; Dube *et al.*, 1985, 1988). Kim and his colleagues (1989) have shown that the contractile responses of vascular smooth muscle induced by $CaCl_2$ and high K^+ may result most likely from increased influx of extracellular Ca^{2+} through the voltage-dependent calcium channels. In terms of these results, the present findings that CUMC6335 inhibited the contraction of rabbit aortic smooth muscle evoked by not only PE (an α_1-adrenergic receptor agonist) but also by high K^+ (a membrane depolarizer) suggest that CUMC6335's vascular relaxation is mediated partly by the blockade of α_1-adrenergic receptors.

In previous studies, three cellular mechanisms have been proposed to explain relaxant response of vascular smooth muscle: (i) blockade of extracellular Ca^{2+} entry into cells (Fleckenstein, 1977; Schwartz and Triggle, 1984), (ii) increase in binding or sequestration of intracellular Ca^{2+} (Watkins and Davidson, 1980, Imai and Kitagawa, 1981), and (iii) inhibiting the release of intracellular stored Ca^{2+} (Imai and Kitagawa, 1981; Ito *et al.*, 1980a, 1980b). In contrast, the contractions of vascular smooth muscles induced by neurohumoral agents have been found to be composed of two components: Phasic contraction induced by the Ca^{2+} released from inside the cell and tonic tension related to the Ca^{2+} influx (Bevan, 1982; Dube *et al.*, 1988), both leading to increased intracellular calcium.

In the light of these findings, it could not be ruled out that CUMC6335 can dilatate the contractile responses of vascular smooth muscle evoked by PE through the blockade of extracellular Ca^{2+} entry into the muscle cells. Thus, these effects of CUMC6335 seem to contribute at least partly to the facts that CUMC6335 reduces blood pressure in rat with renal hypertension (Yokozawa *et al.*, 1994). Extracts of tea (Fitzpatrick *et al.*, 1995) and flavonoids found in tea (Fitzpatrick *et al.*, 1993) have been shown to give vasodilator effects *in vitro*, and higher consumption of black tea was associated with lower SBP (Stensvold *et al.*, 1992). Moreover, 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 PE 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). However, (−) epicatechin's effects are not agreement with the present result that EGCG failed to alter the contractile responses evoked by PE and high potassium in the isolated aortic strips. This finding likely indicates that CUMC6335-induced vasorelaxation is not relevant to the effect of EGCG, which is known to be a main component of catechins derived from green tea leaves. Anyway, these results seem to be similar to those obtained from the isolated rat aortic strips (Lim *et al.*, 2003).

Taken together, these results obtained from the present study suggest that intravenous CUMC6335 causes depressor action in the anesthetized rabbit at least partly through the blockade of adrenergic α_1-receptors. CUMC6335 also causes the relaxation in the isolated aortic strips of the rabbit partly via the blockade of adrenergic α_1-receptors, in addition to the unknown direct mechanism. It seems that there is no species difference in the vascular effect between the rat and the rabbit.

Acknowledgements

This study was supported by a research grant (02-PJ9-PG3-21400-0003) from Health Technology Planning & Evaluation Board, Ministry of Health and Well-being, Korea (2002-2003), and also in part by Grant-in-Aid for Hypertension Research from the Korean Society of Hypertension (2002).

References

Abe Y, Umemura S, Sugimoto K, Hirawa N, Kato Y, Yokoyama N, Yokoyama T, Iwai J, and Ishii M, Effect of green tea rich in gamma-aminobutynic acid on blood pressure of Dahl salt-sensitive rats. *Am J Hypertens* **8**, 74-79 (1995).

Ablad B, Borg KO, Carlsson E, Johnson G, Malmfors L, and Regardh CG. A survey of the pharmacological properties of metoprolol in animals and man. *Acta Pharmacol Toxicol (Copenh)* **36**(5), 7-23 (1975).

Balentine DA, Wiseman SA, and Bouwens LCM, The chemistry of tea flavo-noids. *Crit Rev Food Sci Nut* **37**, 693-704 (1997).

Bevan JA, Selective action of diltiazem on cerebral vascular smooth muscle in the rabbit: antagonism of extrinsic but not intrinsic maintained tone. *Am J Cardiol* **46**, 519-524 (1982).

Bingham SA, Vorster H, Jerling JC, Magee E, Mulligan A, Runswick SA, and Cummings JH. Effect of tea drinking on blood lipids, blood pressure and aspects of bowel habit. *Br J Nutr* **78**, 41-45 (1997).

Bolton TM, Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol Rev* **3**, 606-718 (1979).

Conney AH, Wang ZY, Huang MT, Ho CT, and Yang CS. Inhibitory effect of green tea on tumorigenesis by chemicals and ultraviolet light. *Prev Med* **21**, 361-369 (1992).

Constantine JW, Mcshane WK, Scriabine A, and Hess HJ. Analysis of the hypotensive action of prazosin. In: *Hypertension, Mechanisms and Management* ed. by Onesti G, Kim KE,

Moyer JH, Grume & Stratton Inc, New York, p 429, 1973

De Whalley CV, Rankin SM, Hoult JRS, Jessup W, and Leake DS, Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem Pharmacol* **39**, 1743-1750 (1990).

Dube GP, Baik YH, and Schwartz A, Effects of novel calcium channel agonist dihydropyridine analogue, Bay K 8644, on pig co ronary artery: Biphasic mechanical response and pa radoxical potentiation of contraction by diltiazem and ni modipine. *J Cardiovasc Pharmacol* **7**, 377-389 (1985).

Dube GP, Baik YH, Van Breemen C, and Schwartz A, Effects of isosorbide dinitrate and diltiazem on Ca^{2+} flux and contraction in artery. *European J Pharmacol* **145**, 39-47 (1988).

Fitzpatrick DF, Hirschfield SL, and Coffey RG, Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am J Physiol* **265**, H77-78 (1993).

Fitzpatrick DF, Hirschfield SL, Ricci T, Jantzen P, and Coffey RG, Endothelium-dependent vasorelaxation caused by various plant extracts. *J Cardiovasc Pharmacol* 26, 90-95 (1995).

Fleckenstein A, Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *Ann Rev Pharmacol Toxicol* **17**, 149-166 (1977).

Freis EE, Mackey JD, and Oliver WF, The effect of "sympatholytic" drugs on the cardiovascular responses to epinephrine and norepinephrine in man. *Cir Res* **3**, 254 (1951).

Fujiki H, Yoshizawa S, Horiuchi T, Suganuma M, Yatsunami J, and Nishiwaki S, Anticarcinogenic effects of (−)-epigallocatechin gallate. *Prev Med* **21**, 503-509 (1992).

Graham HN, Green tea consumption and polyphenol chemistry. *Prev Med* **21**, 334-350 (1992).

Henry JP, and Stephens-Larson P, Reduction of chronic psychosocial hypertension in mice by decaffeinated tea. *Hypertension* **6**(3), 437-444, 1984

Hertog, MGL, Feskens EJM, Hollman PCH, Katan MB, and Kromhout D. Dietary antioxidant flavonoids and risk of co ronary heart disease: the Zutphen Elderly Study. *Lancet* **342**, 1007-1011 (1993).

Hodgson JM, Puddey IB, Byrke V, Beilin LJ, and Jordan, N, Effects on blood pressure of drinking green and black tea. *J Hypert* **17**, 457-463 (1999).

Huang Y, Zhang AQ, Lau CW, and Chen ZY, Vasorelaxant effect of purified green tea epicatechin derivatives in rat mesenteric artery. *Life Sci* **63**, 275-283 (1998).

Huang Y, Chan NWK, Lau CW, Yao XQ, Chan FL, and Chen ZY, Involvement of endothelium/nitric oxide in vasorelaxation in duced by purified green tea (−) epicatechin. *Biochim Biophys* Acta **1427**, 322-328 (1999).

Imai S, Kitagawa. A comparison of the differential effects of nitroglycerin, nifedipine, and papaverine on contractures in duced in vascular and intestinal smooth muscle by potassium and lanthanum. *Jap J Pharmacol* **31**, 193 (1981).

Ito Y, Kitamura K, and Kuriyama H, Actions of nitroglycerin on the membrane and mechanical properties of smooth muscles of the coronary artery of the pig. *Br J Pharmacol* **70**, 197-204 (1980).

Ito Y, Kitamura K, and Kuriyama H, Nitroglycerin and catecholamine actions on smooth muscle cells of cannine coronary artery. *J Physiol(London)* **309**, 171-183 (1980).

Keli SO, Hertog MGL, Feskens EIM, and Kromhout D, Dietary flavonoids, antioxidant vitamins, and incidence of stroke. *Arch Intern Med* **156**, 637-642 (1996).

Kim JM, Park KO, and Baik YH, Effects of antiepileptic drugs on contractile responses of vascular smooth muscles. *Chonnam. J Med Sci* **2**(1), 50-59 (1989).

Lim DY, Lee ES, Park HG, Kim BC, Hong SP, and Lee EB, Comparison of Green Tea Extract and Epigallocatechin gallate on Blood Pressure and Contractile Responses of Vascular Smooth Muscle of Rats. *Arch Pharm Res* **26**(3), 214-223 (2003).

Lim DY, Park HG, and Lee BR, Green Tea Extract, not Epigallocatechin gallate Inhibits Catecholamine Release From the Rat Adrenal Medulla. *J Applied Pharmacol* **11**(1), 33-40 (2003).

Pincomb GA, Lovallo WR, Mckey BS, Sung, BH, Passey RB, and Everson SA, Acute blood pressure elevations with caffeine in men with borderline systemic hypertension. *Am J Cardiol* **77**, 270-274 (1996).

Quinlan P, Lane J, and Aspinal L, Effects of hot tea, coffee and water ingestion on physiological responess and mood: role of ca ffeine, water and beverage type. *Psychopharmacology* **134**, 164-173 (1997).

Rakic V, Beilin LJ, and Burke V, Effect of coffee and tea drinking on postprandial hypotension in older men and women. *Clin Exp Pharmacol Physiol* **23**, 559-563 (1996).

Ricc-Evans CA, Miller NJ, Bolwell PG, Bramley PM, and Pridham JB, The relative antioxidant activities of plant-derived po lyphenolic flavonoids. *Free Rad Res* **22**, 375-383 (1995).

Sano M, Takenaka Y, Kojima R, Saito SI, Tomita I, and Katou M, Effects of pu-erh tea on lipid metabolism in rats. *Chem Pharm Bull* **34**, 221-228 (1986).

Schwartz A and Taira N, Calcium channel-blocking drugs: A novel intervention for the treatment of cardiac disease. *Circ Res* (American Heart association Monograph) **52**, 1-183 (1983).

Schwartz A and Triggle DJ. Cellular action of calcium blocking drugs. *Ann Rev Med* **35**, 325-339 (1984).

Stensvold I, Tverdal A, Solvoll K, and Foss OP, Tea consumption. Relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev Med* **21**, 546-553 (1992).

Sung BH, Whitsett TL, Lovallo WR, Absi M, Pincomv GA, and Wilson MF, Prolonged increase in blood pressure by a single orial dose of caffeine in mildly hypertensive men. *Am J Hypertens* **7**, 755-756 (1994).

Tallarida RJ and Murray RB, *Manual of pharmacologic calculations with computer programs*. 2nd ed. Springer-Verlag, New York, p 132, (1987).

Watkins RW and Davidson IWF, Comparative effects of nitroprusside and nitrogiycerin: Actions on phasic and tonic components of arterial smooth muscle contraction. *European J*

Pharmacol **62**, 191-200 (1980).

Wang ZY, Cheng SJ, Zhou ZC, Athar M, Khan WA, and Bickers DR, Antimutagenic activity of green tea polyphenols. *Mutat Res* **223**, 273-285 (1989).

Wang ZY, Huang MT, Ferraro T, Wong CQ, Lou YR, and Reuhl K, Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res* **52**, 1162-1170 (1992).

Yokozawa T, Oura H, Sakanaka S, Ishigaki S, and Kim M, Depressor effect of tannin in green tea on rats with renal hypertension. *Biosci Biotech Biochem* **58**, 855-858 (1994).

Yokogoshi H, Kato Y, Saesaka YM, Takihara-Matsura T, and Takeuchi N, Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Biosci Biotech Biochem* **59**, 615-618 (1995).

Yoshizawa S, Horiuchi T, Fujiki H, Yoshida T, Okuda T, and Sugimura T, Antitumor promoting activity of (−)-epigallocatechin gallate, the main constituent of "tannin" in green tea. *Phytotherapy Res* **1**, 44-47 (1987).

Young W, Hotovec RL, and Romero AG, Tea and atherosclerosis. *Nature* **216**, 1015-1016 (1967).

(Accepted September 15, 2004)