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Comparison of Green Tea Extract and Epigallocatechin Gallate on Blood Pressure and Contractile Responses of Vascular Smooth Muscle of Rats†

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The present study was conducted to investigate the effects of green tea extract (GTE) on arterial blood pressure and contractile responses of isolated aortic strips of the normotensive rats and to establish the mechanism of action. The phenylephrine (10^{-6}~10^{-5} M)-induced contractile responses were greatly inhibited in the presence of GTE (0.3~1.2 mg/mL) in a dose-dependent fashion. Also, high potassium (3.5×10^{-2}~5.6×10^{-2} M)-induced contractile responses were depressed in the presence of 0.6~1.2 mg/mL of GTE, but not affected in low concentration of GTE (0.3 mg/mL). However, epigallocatechin gallate (EGCG, 4~12 μg/mL) did not affect the contractile responses evoked by phenylephrine and high K^+. GTE (5~20 mg/kg) given into a femoral vein of the normotensive rat produced a dose-dependent depressor response, which is transient. Interestingly, the infusion of a moderate dose of GTE (10 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 demonstrate that intravenous GTE causes a dose-dependent depressor action in the anesthetized rat at least partly through the blockade of adrenergic α_1-receptors. GTE also 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. It seems that there is a big difference in the vascular effect between GTE and EGCG.

Key words : Green tea extract, Epigallocatechin gallate, Vasorelaxation, Adrenergic α_1-receptors blockade

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). Interest in the effects of tea has been stimulated by emerging evidence linking tea consumption with positive health outcomes. Recent epidemiological evidence has suggested that tea consumption may be associated with a reduction in heart disease (Hertog *et al.*, 1993) and stroke (Keli *et al.*, 1996). Additionally, evidence from animal models indicates it could act as a cancer preventive agent (Yang and Wang, 1993). These effects may be related to the presence of flavonoids in tea which possess potent antioxidant activity (Rice-Evans *et al.*, 1996) and exert a wide range of other biological activities including anti-inflammatory, anti-allergic and vasodilatory effects (Brandi, 1992; Cook and Samman, 1996).

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

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factors such as blood pressure are of interest. Tea contains caffeine at about 3% of dry weight and polyphenolic compounds at about 40% of dry weight (Harbowy and Balentine, 1997). 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 tea which contains caffeine also induces a transient increase in blood pressure (Quinlan *et al.*, 1997). However, 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*. 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-hour 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 contraction induced by other vasoconstrictors, such as phenylephrine (PE) 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-Alvira and Roche, 1996), possibly via its effects on endothelial function (Briner and Luscher, 1994; Ferro and Webb, 1997; Flavahan, 1992). The main hypothesis tested above studies reported in this paper is that antioxidant (Rice-Evans *et al.*, 1995) and vasodilatory (Fitzpatrick *et al.*, 1993; Fitzpatrick *et al.*, 1995) 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). Pycnogenol contains compounds including catechin, taxifolin, procyanidins of various chain lengths formed by catechin and epicatechin units, and phenolic acids and their glucose esters or glycosides, extracted from the bark of the French maritime pine (Rohdewald, 1997). In contrast to these results, recently 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). 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 green tea extract on blood pressure in the anesthetized rat and contractile responses of isolated aortic strips of the rat and to establish the mechanism of action.

MATERIALS AND METHODS

Experimental procedure

Mature male Sprague-Dawley rats, weighing 150 to 350 grams, 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 rat was anesthetized with thiopental sodium (40 mg/kg) intraperitoneally, 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 (Fig. 1). 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 h, 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 common carotid arteries or 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 rat 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 h 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 h, 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-

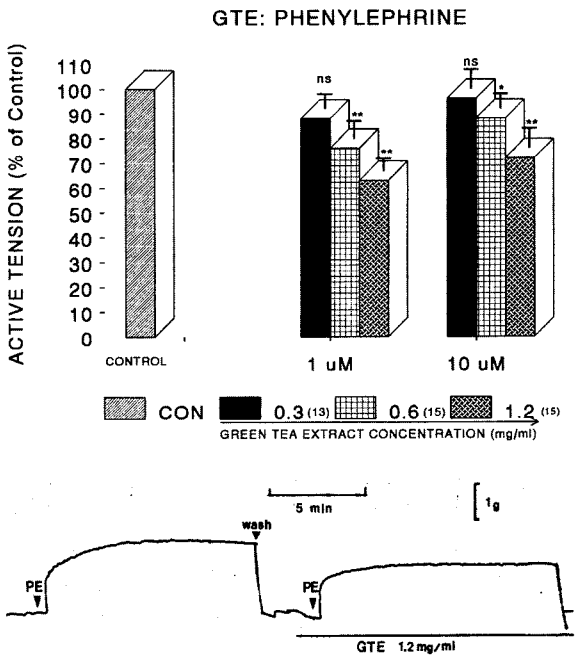

Fig. 1. Upper: Influence of green tea extract (GTE) on phenylephrine (PE)-induced contractile responses in the isolated rat aortic strips. The contractile response was induced by adding 1 μM and 10 μM of PE, respectively after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. "CONTROL" and "AFTER" denote active tension induced evoked by PE before (CONT) and after adding 0.3, 0.6 and 1.2 mg/mL of GTE, respectively. Numeral in the parenthesis indicates number of experimental rat 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 GTE-pretreated group. *: $P<0.05$, **: $P<0.01$. ns: Statistically not significant. **Lower**: The typical tracing showing the effect of green tea extract (GTE) on phenylephrine (PE)-induced contractile responses in the rat aortic strips. Left: PE-induced contractile response. Right: PE-induced contractile response in the presence of 1.2 mg/mL of GTE. At arrow mark, the indicated dose (10^{-5} M) of phenylephrine was added to the bath. The chart speed was 5 mm/min.

gallate and norepinephrine bitartrate (Sigma Chemical Co., U S.A.), thiopental sodium and heparin sodium (Daehan Choorgwae 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 (GTE) on contractile responses induced by Phenylephrine (PE) and high K^+ in the rat aortic strips

The resting tension from the isolated rat aortic strips reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 90 min before the experimental protocol is initiated. The resting tension was adjusted to 0.5 g. The effects of GTE on PE- as well as high K^+ chloride-mediated contractile responses in the rat aorta were examined. In the present study, GTE itself did not produce any effect on the resting tension in the aortic strips isolated from the rat (data not shown). However, it was found that there is difference in the contractile responses induced by PE, but not by high K^+ after pretreatment with GTE as shown in Fig. 2, 3, 4 and 5.

When 10^{-6} M and 10^{-5} M of PE concentrations were administered into the aortic bath in, their active tensions amounted to 2.2±0.2 g and 3.4±0.3 g from the resting tension level, respectively. However, under the pre-existence of GTE at 0.3, 0.6 and 1.2 mg/mL, respectively, 10^{-6} M-PE-induced tensions were dose-dependently inhibited to 88-63% of the control contractile responses, respectively (Fig. 1). Moreover, 10^{-5} M PE-induced contractile responses were also dose-dependently inhibited to 96-72% 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 3.6×10^{-2} M and 5.6×10^{-2} M, which is a membrane-depolarizing concentration, caused an increase in aortic contraction. As shown in Fig. 2, high potassium (3.6×10^{-2} M and 5.6×10^{-2} M)-induced contractile responses after pre-loading with 0.3 mg/mL of GTE did not show any significant difference as compared

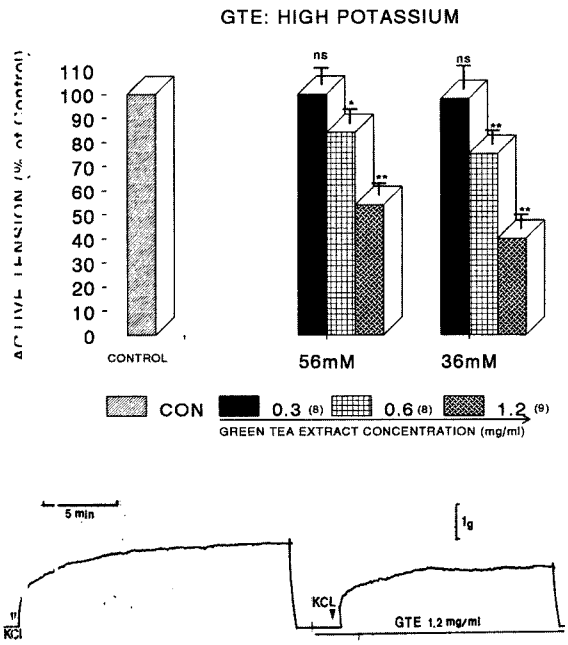

Fig. 2. Upper: Influence of green tea extract (GTE) on high potassium-induced contractile responses in the isolated rat aorta. High potassium (36 and 56 mM, respectively) was added into the bath before and after pretreatment with 0.3, 0.6 1.2 mg/mL of GTE, respectively. Other legends are the same as in Fig. 1. *: P<0.05, **: P<0.01. ns: Statistically not significant. **Lower**: The typical tracing showing the effect of green tea extract (GTE) on high potassium (KCl)-induced contractile responses in the rat aortic strips. Left: KCl-induced contractile response. Right: KCl-induced contractile response in the presence of 1.2 mg/mL of GTE. At arrow mark, the indicated dose (56 mM) of KCl was added to the bath. The chart speed was 5 mm/min.

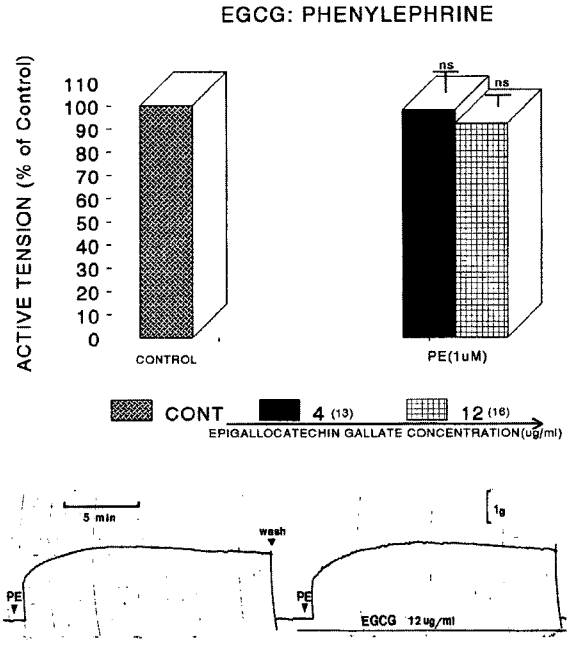

Fig. 3. Upper: Influence of epigallocatechin 3-gallate (EGCG) on phenylephrine (PE)-induced contractile responses in the isolated rat aortic strips. The contractile response was induced by adding 10 μM of PE before (CONTROL) and after adding 4 and 12 μg/mL, respectively. Other legends are the same as in Fig. 1. ns: Statistically not significant. **Lower**: The typical tracing showing the effect of epigallocatechin 3-gallate (EGCG) on phenylephrine (PE)-induced contractile responses in the rat aortic strips. Left: PE-induced contractile response. Right: PE-induced contractile response in the presence of 12 μg/mL of EGCG. At arrow mark, the indicated dose (10^{-5} M) of phenylephrine was added to the bath. The chart speed was 5 mm/min.

with their corresponding control responses, respectively. However, in the presence of GTE at concentrations of 0.6 and 1.2 mg/mL, high potassium (3.6×10^{-2} M and 5.6×10^{-2} M)-induced contractile responses were 84-40% of their corresponding control responses in a dose-dependent fashion, respectively (Fig. 2).

Effects of epigallocatechin 3-gallate (EGCG) on contractile responses induced by PE and high K^+ in the rat 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 4 and 12 μg/mL of EGCG, the aortic contractile response evoked by PE (10^{-6} M) was 98-94% of the control, respectively in comparison with the corresponding control response (2.3±0.2 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 4 and 12 μg/mL of EGCG it amounted to 95-91% of the corresponding control response, which there was no statistically difference between control and EGCG-treated groups (Fig. 4).

Effects of green tea extract (GTE) and epigallocatechin-3-gallate (EGCG) on norepinephrine-induced hypertensive responses

Since GTE greatly inhibited PE-induced contractile responses of the isolated aortic smooth muscle as shown in Fig. 2, it is of interest to examine the effect of intravenous GTE 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 14 rats, norepinephrine at doses of 0,3, 1.0 and 3.0 μg/kg caused dose-dependent pressor responses of 15±3 mmHg, 20±3 mmHg and 36±5 mmHg from the original baseline (122±15 mmHg), respectively. However, after infusion of GTE with a rate of 10 mg/kg/30 min, they were significantly depressed to 8±1 mmHg ($P< 0.01$), 12±2 mmHg ($P<0.01$) and 18±4 mmHg ($P< 0.01$) at

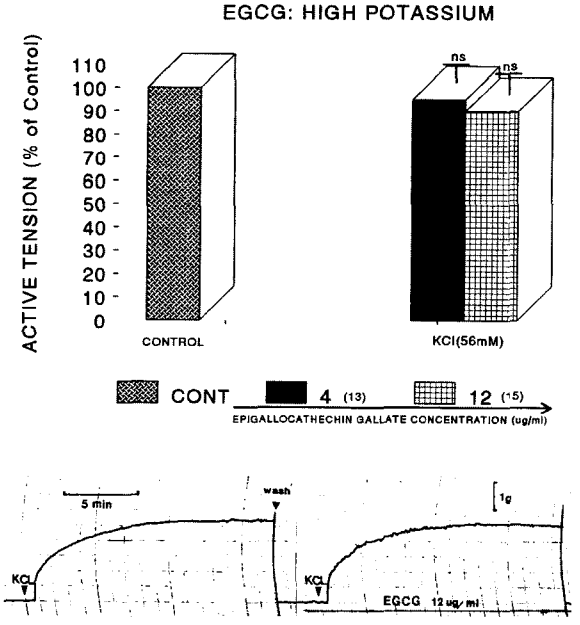

Fig. 4. Upper: Influence of epigallocatechin 3-gallate (EGCG) on high potassium (KCL)-induced contractile responses in the isolated rat aortic strips. The contractile response was induced by adding 56 mM of KCl before (CONTROL) and after adding 12 μg/mL of EGCG. Other legends are the same as in Fig. 1. ns: Statistically not significant. **Lower**: The typical tracing showing the effect of epigallocatechin 3-gallate (EGCG) on high potassium (KCl)-induced contractile responses in the rat aortic strips. Left: KCl-induced contractile response. Right: KCl-induced contractile response in the presence of 12 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.

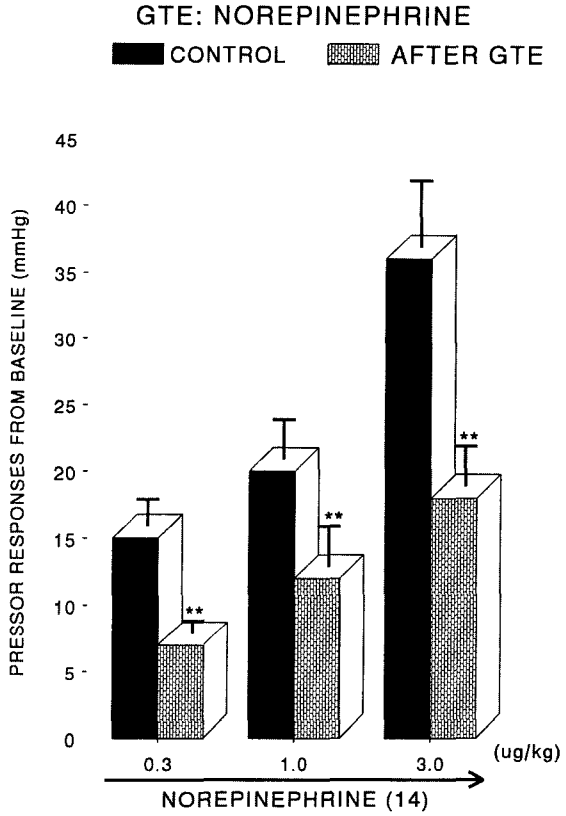

Fig. 5. Influence of intravenous green tea extract on arterial blood pressure in anesthetized rats. Ordinate: Changes of blood pressure from baseline level in mmHg from 8 rats. Abscissa: Intravenous doses of green tea extract in mg/kg. Vertical bar on top of each column indicates standard error of mean (S.E.M.). There was statistically significant difference in changes of arterial pressure responses induced by green tea extract. Numeral in the parenthesis denotes number of animals used in the experiment. The original base-line of arterial blood pressure was 121±20 mmHg. **: $P<0.01$.

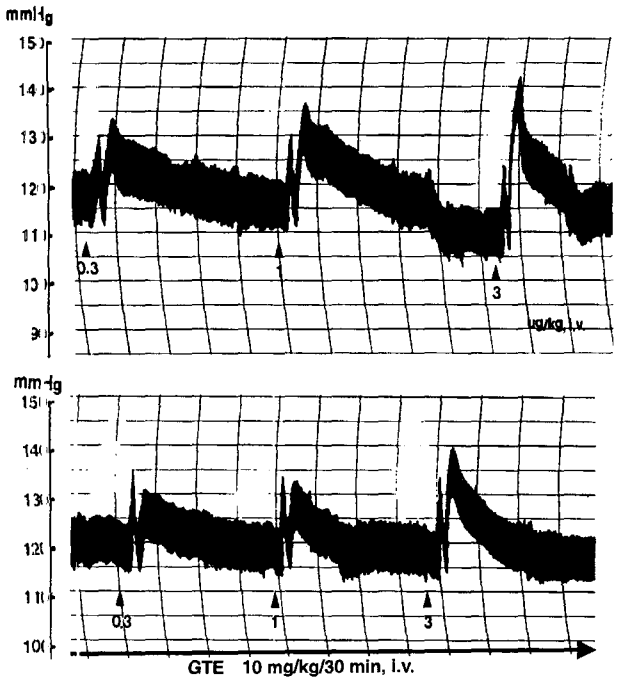

Fig. 6. The representative tracing of green tea extract (GTE) effect on intravenous norepinephrine (NE)-induced pressor response in the anesthetized. At arrow marks, the indicated doses (0.3, 1.0 and 3.0 μgkg) of NE were administered into a femoral vein. Upper: NE only-induced hypertensive responses in a non-treated rat. Lower: NE-induced hypertensive responses in the GTE-pretreated rat. GTE was infused into a femoral vein with a rate of 10 mg/kg/30 min. ABP: arterial blood pressure in mmHg. The chart speed was 20 mm/min.

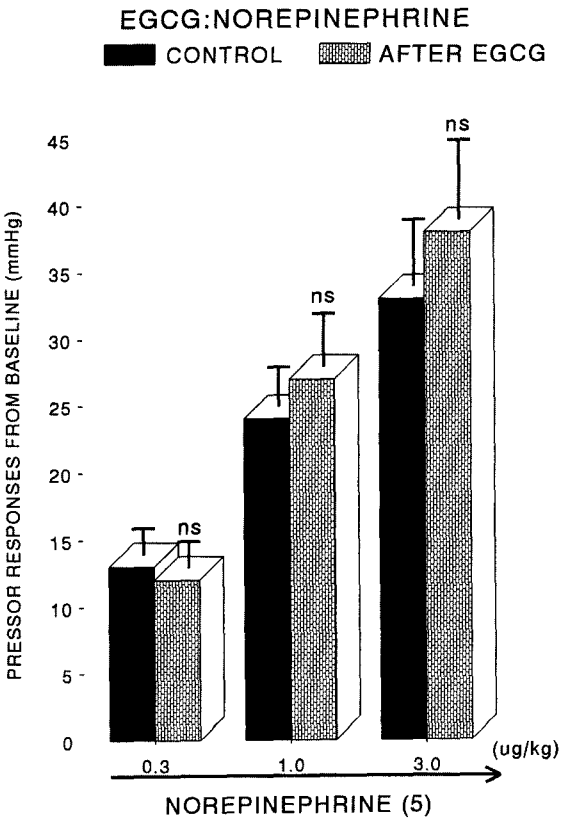

Fig. 7. Influence of intravenous epigallocatechin 3-gallate (EGCG) on norepinephrine-evoked pressor responses. EGCG was infused into a femoral vein with a rate of 1 mg/kg/30 min after obtaining the corresponding control responses of intravenous norepinephrine. Other legends are the same as in Fig. 5. ns: Statistically not significant.

the above same doses, respectively (Fig. 6 and 7). Fig. 6 shows that norepinephrine-evoked pressor responses are greatly attenuated after pretreatment with intravenous GTE.

In order to compare to GTE effects, it was tried to examine the effect of EGCG on norepinephrine-evoked pressor responses. In 5 rats, norepinephrine at doses of 0,3, 1.0 and 3.0 μg/kg before the pretreatment with EGCG caused dose-dependent pressor responses of 13±2 mmHg, 24±3 mmHg and 33±5 mmHg from the original baseline, respectively. However, after infusion of EGCG with a rate of 1 mg/kg/30 min, they were 12±2 mmHg (ns), 27±4 mmHg (ns) and 38±6 mmHg (ns) at all the above same doses, respectively (Fig. 7 and 8). Fig. 8 shows that norepinephrine-evoked pressor responses are not affected after pretreatment with intravenous EGCG.

DISCUSSION

The present experimental results demonstrate that intravenous GTE causes a dose-dependent depressor action in the anesthetized rat at least partly through the blockade of adrenergic α_1-receptors. It seems that GTE also causes vascular relaxation in the isolated aortic strips of the rat via the blockade of adrenergic α_1-receptors. However, EGCG did not. This indicates that there is a big difference in the vascular effect between GTE and EGCG.

In support of these data, 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 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 GTE can cause the depressor effect.

In general, among drugs which interfere with peripheral sympathetic function, adrenergic α-receptor blocking agents

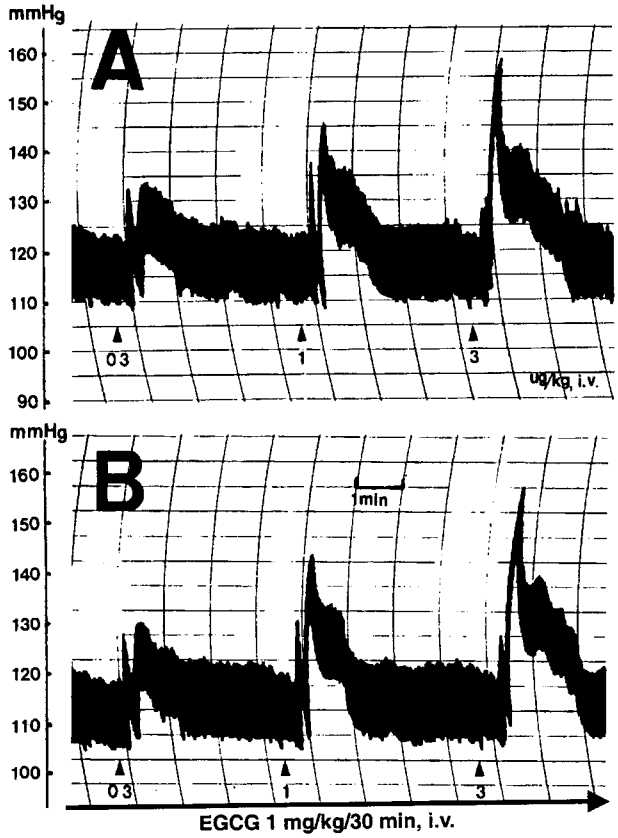

Fig. 8. The representative tracing of epigallocatechin 3-gallate (EGCG) effect on intravenous norepinephrine (NE)-induced pressor response in the anesthetized. At arrow marks, the indicated doses (0.3, 1.0 and 3.0 μgkg) of NE were administered into a femoral vein. Upper: NE only-induced hypertensive responses in a non-treated rat. Lower: NE-induced hypertensive responses in the EGCG-pretreated rat. EGCG was infused into a femoral vein with a rate of 1 mg/kg/30 min. ABP: arterial blood pressure in mmHg. The chart speed was 20 mm/min.

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 epinephrine 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.*, 1975) 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 GTE, it is thought that GTE has vascular dilatatory activity through the adrenergic α-receptor blockade. In view of these reports, in the present work, the finding that GTE attenuated the norepinephrine-induced pressor responses demonstrates that GTE 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; Fitzpatrick *et al.*, 1995) polyphenolics in tea can attenuate the transient pressor effect of caffeine, and lower blood pressure during regular consumption. Recently, 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 norepinephrine. EGCG is well known to be a major component of catechins found in green tea. This finding suggests that GTE-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 GTEs vasorelaxation is not associated to the effects of catechins including EGCG contained in GTE. 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 (1992; Fujiki *et al.*, 1992; Wang *et al.*, 1992). Moreover, animal experiments have also studied the protective effects of green tea against cardiovascular diseases (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 & 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 GTE inhibited the contraction of rat aortic smooth muscle evoked by not only PE (an α_1-adrenergic receptor agonist) but also by high K^+ (a membrane depolarizer) indicate that GTEs vascular relaxation is mediated 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 (Fleckstein, 1977; Schwartz & Triggle, 1984), (ii) increase in binding or sequestration of intracellular Ca^{2+} (Watkins & Davidson, 1980; Imai & Kitagawa, 1981), and (iii) inhibiting the release of intracellular stored Ca^{2+} (Imai & Kitagawa, 1981; Ito *et al.*, 1980a, b). 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 GTE 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 GTE seem to contribute at least partly to the facts that GTE 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, (-) epicatechins 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 GTE-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.

Collectively, these experimental results demonstrate that intravenous GTE causes a dose-dependent depressor action in the anesthetized rat at least partly through the blockade of adrenergic α-receptors. GTE also causes vascular relaxation in the isolated aortic strips of the rat via the blockade of adrenergic α_1-receptors. However, EGCG did not. It seems likely that there is much difference in mode of action between GTE and EGCG.

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