Increases in Blood Pressure and Heart Rate Induced by Caffeine are Inhibited by (-)-Epigallocatechin-3-O-gallate: Involvement of Catecholamines

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Abstract: In a previous experiment, (-)-epigallocatechin-3-O-gallate (EGCG) reduced caffeine-induced locomotor activity and stereotyped behaviours and inhibited caffeine-induced neuronal stimulant activity. This research was performed to give additional evidence that EGCG counteracts caffeine-induced stimulant effects in animals. EGCG inhibited caffeine-induced cardiovascular activation measures, such as arterial pressure (AP) and heart rate (HR). In addition, the increases in the levels of adrenaline and noradrenaline in the blood induced by caffeine were reduced by EGCG. We suggest that EGCG may reduce caffeine-induced increases in blood pressure and HR and may decrease catecholamines levels in the blood. Therefore, EGCG counteracts caffeine-induced cardiovascular activity. The stimulant effects of caffeine should be reduced by the amount of EGCG in green tea.

Key words: (-)-epigallocatechin-3-O-gallate (EGCG), caffeine, catecholamines, arterial pressure (AP), heart rate (HR)
INTRODUCTION

Caffeine and (-)-epigallocatechin-3-O-gallate (EGCG) are major active components in green tea (Camellia sinensis). Green tea contains caffeine (1-5%) with small amounts of other xanthine alkaloids. The EGCG content of green tea is approximately 3%. Although tea composition varies with climate, season, tea variety, and age of the leaf, caffeine and EGCG generally are present in green tea at similar concentrations 1-3.

Caffeine enhances dopaminergic and noradrenergic neuronal activities by competitive antagonism of adenosine receptors that are co-localised and functionally interact with dopaminergic and adrenergic receptors in the central and peripheral nervous systems 4-5. Caffeine produces psychomotor stimulant effects similar to those of amphetamine and cocaine, increasing locomotor activity and arousal, and stimulating cardiovascular functions. Therefore, the behavioural profile of caffeine is similar to those of amphetamine and cocaine. Caffeine affects the cardiovascular systems by directly modifying the contractility of the musculature in the heart and blood vessels as well as by influencing neurotransmission peripherally and centrally 6. Caffeine was found to induce, to various degrees, dose-dependent early increases in HR and systolic–diastolic AP, and to increase the concentration of cardiac catecholamines in rats and guinea pigs 7-8.

Recently, we reported that EGCG and caffeine in green tea have opposite pharmacological effects; EGCG inhibited caffeine-induced hyperactivity via a dopaminergic blockade, and reduced the anxiogenic-like effects induced by caffeine 9-10. Therefore, we suggested that the stimulant effects of caffeine could be inhibited by EGCG in green tea. Based on previous reports, we measured cardiovascular responses after concomitant administration of caffeine and EGCG. We are interested in whether the increases in AP and HR induced by caffeine can be reduced by EGCG. In addition, the concentrations of catecholamines such as adrenaline, noradrenaline and dopamine in the blood were measured to determine possible mechanisms.

METHODS
**Subjects:** Groups of 7-8 male Wistar rats (Samtako, Suwon, Korea) weighing 250-350 g were used for all experiments. Animals were housed in acrylic cages with water and food available *ad libitum* under an artificial 12-h light/dark cycle (light on at 7:00 am) and at a constant temperature (22±2°C). To ensure adaptation to the new environment, rats were kept in the departmental holding room for 1 week before testing. Rats were fasted for 24 h before the injection of pentobarbital. All of the experiments were conducted in accordance with the guidelines for the care and use of laboratory animals of the National Institute of Toxicological Research on the Korea Food and Drug Administration. Anhydrous caffeine (Nakarai Chemicals, Ltd. Kyoto, Japan), EGCG (Sigma, St. Louis, MO, USA), pentobarbital sodium (Hanlim Pharm. Co., Ltd. Seoul, Korea) and the Catecholamines (adrenaline/noradrenalin/dopamine) Enzyme Immunoassay kit (Labor Diagnostika Nord GmbH & Co., Nordhorn, Germany) were used in this experiment. All the other chemicals used in these experiments were obtained from Sigma (St. Louis, MO, USA). EGCG (purity ≥95 %) and caffeine were dissolved in saline just before the experiment. Rats were intraperitoneally (i.p.) treated with EGCG (15 and 30 mg/kg) and caffeine (25 mg/kg) simultaneously because we confirmed that the above doses also were reasonable in the preliminary experiment and in our previous experiments 9-10.

**Procedure:** The AP was continuously recorded in conscious unrestrained rats 11-12. Briefly, the rats were anesthetised with a mixture of ketamine (100 mg/kg, i.p.) and acepromazine (5 mg/kg, i.p.). The catheter (PE-10 connected to PE-50) was inserted into the aorta and then tunnelled subcutaneously and exteriorised through the interscapular skin. After 7 d of recovery, the aortic catheter was connected to a pressure transducer via a rotating swivel that allowed the rat to move freely in the cage. After approximately 3 h of habituation, the AP signal was digitised by a microcomputer for 2 h (12:00-14:00 h). Systolic and diastolic AP values were determined on-line. Using off-line analysis, the mean values of these parameters were calculated for a period of 2 h, and these mean values served as systolic and diastolic AP. Each rat was implanted with a transmitter (Data Sciences International TA11CTA-F40, St. Paul, MN, USA) for electrocardiography (EEG) 13-15. The paired-wire electrodes for the precordial bipolar lead (apex-base lead) were placed at the cervical subcutaneous region over the trapezius, and the skin was closed by suture. All surgical procedures
were performed stereotaxically under aseptic conditions. Surgical anaesthesia was performed with pentobarbital (50 mg/kg, i.p.), and all efforts were made to minimise the suffering of the animals. Seven days post-surgery, the rats were divided into control and treatment groups (7-8 rats in each group). The signals were processed by a Data Sciences International analogue converter and routed to an AD converter (Eagle PC30, USA) housed in a PC class computer. The AD converter digitised the EEG and activity signals at 128 Hz. The digitised data were transferred to the computer and displayed graphically by the program on the computer monitor. The HR was continuously recorded for 10 min at the same time. Data for a 10 min segment were extracted from each period. After collecting the data, the HR was extracted using the analysis software Dataquest A.R.T. 4.1 (Data Sciences International, St. Paul, MN, USA) using the interbeat interval (IBI) from the marked waveform.

Plasma catecholamines levels (adrenaline/noradrenalin/dopamine) were measured using a competitive enzyme immunoassay kit. Blood was collected via heart puncture of the rats 22-23 min after treatment with caffeine using a 1.2×38 mm bevelled needle (BD Discardit™, NJ, USA) and a 5 ml syringe (Doowon Meditec, Yongin, Korea) containing EDTA (ethylenediaminetetraacetic acid, final concentration: 1 mM) as an anticoagulant. The blood samples were centrifuged at 1,000 × g for 10 min at 4 °C. Blood samples were maintained at 2 °C until analysis.

Data were presented as the mean ± SEM. For the statistical comparison, the results were analysed using a one-way analysis of variance (ANOVA). A P-value * < 0.05 was considered statistically significant. In cases of significant variation, the individual values were compared with the Holm-Sidak test.

RESULTS

Effects of EGCG on caffeine-induced mean AP and HR

The mean AP and HR were observed for 30 min in the rats treated only with caffeine alone. The mean AP and HR transiently increased 20-23 min after administration. The increases in mean AP and HR induced by caffeine were decreased by the concomitant administration of EGCG in a dose-
dependent manner with respect to the control level (Fig. 1). However, EGCG (15 and 30 mg/kg) alone did not affect the mean AP and the heart rate (data not shown).

**Effects of EGCG on catecholamines levels after caffeine administration**

The plasma concentrations of adrenaline and noradrenaline were significantly reduced by concomitant administration of EGCG (15 and 30 mg/kg) 22-23 min after administration; no reduction was observed for dopamine. (Fig. 2).

**DISCUSSION**

Caffeine, the most widely consumed central nervous system stimulant, exerts obvious effects on anxiety and sleep that vary according to individual sensitivity to methylxanthines 19-20. As caffeine is consumed by people in a variety of foods, beverages and over-the-counter medications, excess caffeine consumption can often cause insomnia at night. It is believed that people who drink coffee have more difficulty falling asleep at night than those who drink green tea. It is notable that there are pharmacological differences between drinking coffee and green tea even though there are similar levels of caffeine in a cup of green tea and a cup of coffee. In the previous study, we confirmed that EGCG inhibits caffeine-induced hyperactivity and reduced caffeine-induced anxiety, demonstrating that caffeine-induced pharmacological effects can be counteracted by EGCG 9.

Based on previous results, we are also interested in whether EGCG reverses caffeine-induced cardiovascular responses such as increased mean AP and HR 21-22. The ingestion of caffeine and green tea results in a transient increase in AP in subjects who have avoided caffeine for 12 h or more 23-24. Moreover, it has been shown that (-)-epicatechin also reduces arterial contraction induced by other vasoconstrictors such as phenylephrine and endothelin-1 25. It has been also found that (-)-epicatechin can act on the endothelium to increase the intracellular Ca^{2+} concentration and to induce nitric oxide release, which may account for the endothelium-dependent relaxation in isolated rat mesenteric arteries 26. We also found that EGCG decreased the increases of mean AP and HR induced by caffeine.
In addition, the concentrations of catecholamines such as noradrenaline and adrenaline were measured in the blood. EGCG also suppressed the increases in noradrenaline and adrenaline levels induced by caffeine. Cardiovascular tone is regulated by adrenergic systems. Because catechin-polyphenols are known to be capable of inhibiting catechol-O-methyl-transferase (the enzyme that degrades noradrenaline) and caffeine is known to inhibit transcellular phosphodiesterases (enzymes that break down noradrenaline-induced cAMP), it has been 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 noradrenaline-cAMP axis. More recently, it has been found that catechin-polyphenols in green tea extract induce relaxation in the isolated aortic strips from rats via the blockade of adrenergic α₁-receptors, in addition to an unknown direct mechanism. It also inhibits catecholamines secretion evoked by stimulation of cholinergic nicotinic receptors as well as the direct membrane depolarisation from isolated perfused rat adrenal glands.

Consequently, catecholamines are important in controlling peripheral vascular responses. Adrenaline is a powerful cardiac stimulant, accelerating the rate of spontaneous beating. In addition, adrenaline also evokes a characteristic effect on mean AP, which increases rapidly to a peak that is proportional to the level in the blood. On the other hand, noradrenaline increases the diastolic and systolic pressures. Accordingly, the increase in the mean mean AP is attributed to both adrenaline and noradrenaline. However, adrenaline plays a more important role in the increase in the HR.

In conclusion, this study provides additional evidence that EGCG counteracts caffeine-induced stimulant responses of cardiovascular systems, showing that the decrease in catecholamines plays an important role in this effect. In addition, EGCG which is present in green tea may decrease the stimulant cardiovascular responses of caffeine. Thus, it implies that a cup of green tea will have less stimulant effects than a cup of coffee containing the same amount of caffeine.

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REFERENCES


Figure Legends

Fig. 1. Effects of (-)-epigallocatechin-3-O-gallate (EGCG) on heart rate (HR) (A) and mean arterial pressure (AP) (B) after caffeine administration. Twenty-two minutes after intraperitoneal administration of caffeine (25 mg/kg) and EGCG (15 and 30 mg/kg) to rats (n=5 or 6/group), the HR and AP were measured. Each column represents the mean with the S.E.M. The levels of significance are *P<0.05 and ***P<0.001 relative to the caffeine group and ###P<0.001 relative to the untreated group.

Fig. 2. Effects of EGCG on plasma catecholamine levels after caffeine administration. Twenty-two minutes after intraperitoneal administration of caffeine (25 mg/kg) and EGCG (15 and 30 mg/kg) to rats (n=5 or 6/group), the CA levels were measured. Each column represents the mean with the S.E.M. The levels of significance are *P<0.05 and ***P<0.001 relative to the caffeine group and ###P<0.001 relative to the untreated group.
Fig 2