

Green Tea Catechins Increase the Force of Contraction in Isolated Guinea Pig Atrial Muscle Preparations by Increasing the Amplitude of Intracellular Ca^{2+} Concentration

Takushi SASAKI¹⁾, Ryo KAMATA^{1)*}, Shunji UENO²⁾, Takeharu KANEDA³⁾ and Kyosuke TEMMA¹⁾

¹⁾Laboratory of Toxicology, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan

²⁾Laboratory of Veterinary Public Health, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan

³⁾Laboratory of Veterinary Pharmacology, Nippon Veterinary and Life Science University, Musashino, Tokyo 180-8602, Japan

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ABSTRACT. It has been reported that green tea catechins enhance the force of contraction of isolated heart muscle preparations. However, it remains controversial whether or not the increase in force of contraction is related to an increase in the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). In this study, the relationship was investigated using a left atrial muscle preparation isolated from guinea pig heart. In the left atrial muscle preparations without fura-2/AM loading, neither EGC (epigallocatechin) nor EC (epicatechin) influenced the force of contraction, but EGCG (epigallocatechin gallate) and ECG (epicatechin gallate) increased the force of contraction in a dose-dependent manner. The ED_{50} value of EGCG was significantly higher than that of ECG. In the atrial muscle preparations loaded with fura-2/AM, EGCG and ECG increased the amplitude of $[\text{Ca}^{2+}]_i$ (peak $[\text{Ca}^{2+}]_i$ minus diastolic $[\text{Ca}^{2+}]_i$) which is associated with the increase in force of contraction. Simple regression analysis between the degree of increase in the force of contraction and the increase in the amplitude of $[\text{Ca}^{2+}]_i$ revealed a positive correlation in EGCG, ECG and CaCl_2 . In addition, the slopes of the regression lines of EGCG and ECG were comparable with those of CaCl_2 . It was suggested that atrial muscle preparations had a higher affinity for ECG than EGCG, and that the increase in the force of contraction by EGCG and ECG was closely related to the increase in the amplitude of $[\text{Ca}^{2+}]_i$.

KEY WORDS: epicatechin gallate, epigallocatechin gallate, force of contraction, intracellular Ca^{2+} concentration, isolated guinea pig heart.

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It has long been considered that drinking green tea is beneficial to human health, in particular, prevention of malignant tumors and cardiovascular disease [1, 5, 10, 16]. About 30% of green tea is composed of polyphenol compounds, and about 80% of these polyphenols belong to the catechin group. Recently, the biological activity of the catechin compounds has been explored, and identification of the mechanisms involved in their health benefits has attracted attention.

Previously, Kubota *et al.* [7] reported that EGCG, one of the catechins, increased the force of contraction in an isolated rat atrial muscle preparation. Research into whether or not the increase in force of contraction caused by EGCG is related to the increase in $[\text{Ca}^{2+}]_i$ has been contradictory; experimental results reported by three main research groups were inconsistent. Lorenz *et al.* [9] reported that an increase in the force of contraction of EGCG was always accompanied by an increase in $[\text{Ca}^{2+}]_i$ in rat cardiac myocytes. However, Li *et al.* [8] reported that EGCG did not cause any increase in $[\text{Ca}^{2+}]_i$ in rat cardiac myocytes, at any of the concentrations which produced concentration-dependent increases in the force of contraction. Hotta *et al.* [6] re-

ported that an increase in $[\text{Ca}^{2+}]_i$ was observed only at a low concentration of EGCG and not at a higher concentration, even when marked increases in the force of contraction by EGCG were observed in guinea pig heart muscle preparations. Therefore, the underlying mechanism remains controversial.

To investigate the different experimental results, an isolated guinea pig left atrial preparation loaded with fura-2/AM was used, and changes in the force of contraction by EGCG and changes in $[\text{Ca}^{2+}]_i$ were simultaneously recorded in this study. The left atrial muscle preparation showed stable contractions during repeated electrical stimulation throughout a relatively long experiment [4]. In addition, since the muscle tissue is sufficiently thin and the size is appropriate for measurement of $[\text{Ca}^{2+}]_i$, this experiment has the advantage that simultaneous recordings of the force of contraction are possible from the same area of the myocardial tissue with only a small variation in experimental results. In this study, EC, EGC and ECG (Fig. 1), also contained in green tea, were employed, to determine the relationship between the structures of catechins and their actions on the force of contraction and $[\text{Ca}^{2+}]_i$.

MATERIALS AND METHODS

Animals: Male Hartly guinea pigs obtained from Japan SLC Inc. (Hamamatsu, Japan) were used. The animals were housed 3 per cage and maintained in a room with a constant

*CORRESPONDENCE TO: KAMATA, R., Laboratory of Toxicology, School of Veterinary Medicine, Kitasato University, Higashi 23-bancho, Towada, Aomori 034-8628, Japan.
e-mail: kamata@vmas.kitasato-u.ac.jp

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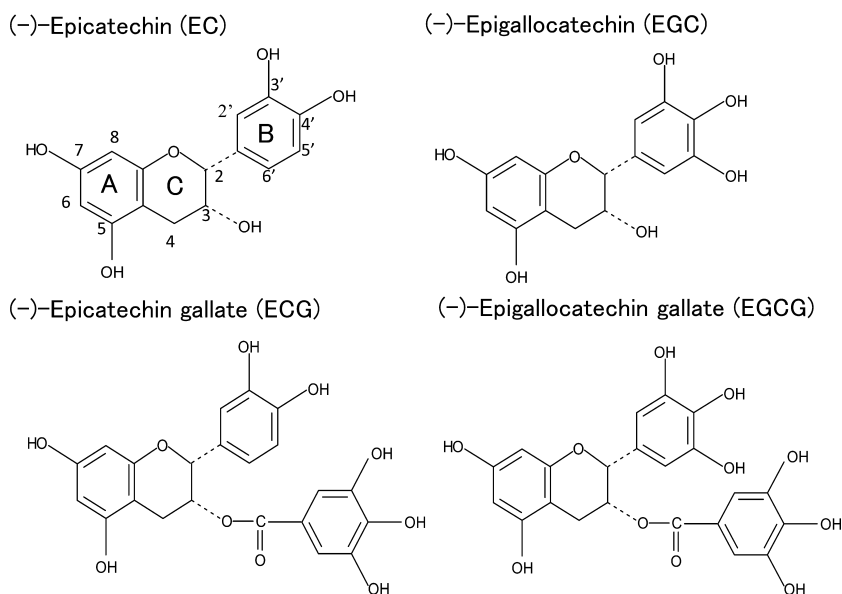


Fig. 1. Structure of catechins.

temperature ($25 \pm 1^\circ\text{C}$), humidity ($60 \pm 10\%$) and lighting conditions (the lights were on between 8 a.m. and 8 p.m.), and were fed a standard laboratory chow.

All protocols in this study were approved by the Institutional Animal Care and Use Committee at Kitasato University and in accordance with the Guide for the Use of Laboratory Animals issued by the United State Department of Health and Human Services.

Isolation of fura-2/AM unloaded left atrial muscle preparations and measurements of the force of contraction: Male Hartly guinea pigs (350–450 g) were sacrificed by cervical dislocation. The hearts were rapidly removed and perfused with a modified Krebs-Henseleit bicarbonate buffer solution (NaCl, 118 mM; NaHCO_2 , 27.2 mM; KCl, 4.8 mM; KH_2PO_4 , 1.0 mM; CaCl_2 , 1.2 mM; MgSO_4 , 1.2 mM; glucose, 11.1 mM) through the aorta for 5 min, using Langendorff apparatus, to remove visible blood. The buffer solution was saturated with a 95% O_2 /5% CO_2 gas mixture and maintained at 30°C (pH 7.4). After all visible blood was removed from the heart, strips of the left atrial muscle were excised and vertically suspended in a chamber containing 20 ml of the above oxygenated buffer solution. The atrial muscle preparations were electrically stimulated at 2 Hz with square-wave pulses of 3 msec duration at a voltage approximately 30% above the threshold. A pair of platinum electrodes was used for electrical-field stimulation [3]. The resting tension was adjusted to 1.0 g. The isometric force of contraction was continuously recorded using a pen recorder (WI 621G; Nihon Kohden, Tokyo, Japan) and a Power Lab 4/20 computer system (ADI Instruments, Colorado Springs, CO, U.S.A.). After a 90 to 120 min equilibration period under the experimental conditions, the force of contraction was relatively stable for the next 60 min. Ascorbic acid, 0.1 mM, was added to the incubation solution to depress oxidation

of EGCG, ECG, EGC or EC. Then, one of these catechins was added to the incubation solution 2 min later to observe the concentration-dependent inotropic effect. The concentration of the catechin was increased cumulatively at 20 min intervals or when the effects reached a steady state at each concentration. Ascorbic acid, 0.1 mM, did not affect the force of contraction of the left atrial muscle preparations.

Isolation of fura-2/AM loaded left atrial muscle preparations: After the hearts were perfused with bicarbonate buffer solution using the Langendorff apparatus, described above, at a constant flow rate of 5 ml/min at 30°C for 30 min, the perfusion was switched to re-circulating using the buffer solution containing 2 μM fura-2/AM and 0.025% cremophor EL. The hearts were perfused with the re-circulating solution for 60 min, and then perfused with fura-2/AM and cremophor EL-free buffer solution for 15 min to remove fura-2/AM from the extracellular space. The whole left atrial muscle was excised, and the muscle layer of the atrial muscle on the ventricular muscle side was cut off and discarded. Then, only the muscle layer on the pericardial side was used as the left atrial muscle preparation for simultaneous measurement of force of contraction and $[\text{Ca}^{2+}]_i$.

Simultaneous measurements of force of contraction and $[\text{Ca}^{2+}]_i$: Fura-2/AM loaded left atrial muscle preparations were horizontally suspended in a thin quartz glass bottom chamber containing 2 ml of the above oxygenated buffer solution, and were perfused at flow rate of 3 ml/min at 30°C . The atrial muscle preparations were stimulated at 2 Hz using a pair of platinum electrodes for electrical-field stimulation at the 1.0 g resting tension. The isometric force of contraction was continuously recorded using a pen recorder and a Power Lab 4/20 computer system, as described above.

Fura-2 fluorescence was measured with a dual excitation fluorometer (CAM 230, Jasco, Tokyo, Japan). Emission of

the excitation light at 340 and 380 nm to the left atrial muscle preparation and detection of the intensity of the fluorescence at 500 nm were carried out via the optic fiber (FB 30, Jasco, Tokyo, Japan) connected to the fluorometer. The luminous flux at the tip of the optic fiber was 7 mm in diameter, and the size corresponded to the surface area of the left atrial muscle preparation: it was possible to record fluorescence signals from the whole muscle preparation. Therefore, changes in $[Ca^{2+}]_i$ were simultaneously recorded with the force of contraction from the same muscle tissue area. The simultaneous recording system was similar to the system reported by Miyamoto *et al.* [11].

The ratio of fluorescence intensities at the excitation wavelengths of 340 and 380 nm was continuously recorded for about 60 min during the experiment and analyzed with the fluorometer. The analyzed data were then recorded and stored in a pen recorder and the Power Lab 4/20 computer system. Under these experimental conditions, despite the constant force of contraction, the amplitude of $[Ca]_i$ gradually decreased at a constant rate for 30 min. Obtained values were subjected to the following formula ($y = -0.52x + 99.9$, $n=5$; y , amplitude of $[Ca^{2+}]_i$; x , elapsed time; $r=0.998$).

The amplitude of $[Ca^{2+}]_i$ was calculated by subtraction of the value of the 340/380 nm ratio at the maximal diastolic period from the value of the 340/380 nm ratio at the peak of $[Ca^{2+}]_i$ [4]. In the experiments with EGCG (20, 100, and 300 μ M) and ECG (5 and 50 μ M), the force of contraction and $[Ca^{2+}]_i$ were examined in one muscle preparation using one concentration of each catechin. In the experiments with $CaCl_2$, it was cumulatively increased from 1.2 to 1.8, 3.0 and 4.8 mM in one muscle preparation, and dose-dependent changes in the force of contraction and $[Ca^{2+}]_i$ were recorded (when a peak of the force of contraction at each $CaCl_2$ concentration was observed, the next higher concentration was used. The interval was about 2–3 min).

To depress the oxidation of the catechins, 0.1 mM ascorbic acid was added to the incubation solution 2 min before catechin addition. This concentration of ascorbic acid affected neither the force of contraction nor the $[Ca^{2+}]_i$.

Chemicals and statistical analyses: Fura-2/AM was purchased from Dojindo Laboratories (Kumamoto, Japan). (–) EC, (–) EGC, (–) EGCG and (–)ECG were purchased from Sigma (St. Louis, MO, U.S.A.). Cremophor EL was purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals were of reagent grade.

Pearson's correlation coefficient analysis and simple regression were used to assess the relationship between force of contraction and amplitude of $[Ca^{2+}]_i$. Differences in the correlation coefficients of the regression lines obtained from $CaCl_2$, EGCG and ECG were determined by testing the t -value.

Other data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. When appropriate, Student's unpaired t -test was used. The criterion for statistical significance was a P value of less than 0.05. All values are expressed as a mean \pm SE.

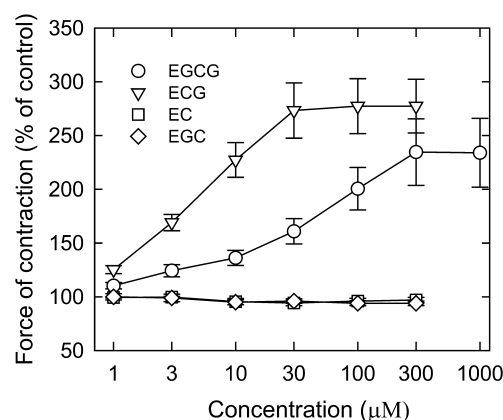


Fig. 2. Effects of EGCG, ECG, EGC and EC on force of contraction in guinea pig left atrial muscle preparations. Left atrial muscle preparations were incubated in a chamber contained 20 ml Krebs-Henseleit bicarbonate buffer solution under 2 Hz stimulation rate at 30°C. Values are expressed as percentage of initial values (set at 100%) observed immediately before the addition of catechins. Each point represents the mean of 4 to 6 experiments. Vertical lines indicate the standard error of the mean. Open diamond, EGC; open triangle, ECG; open square, EC; open circle, EGCG.

RESULTS

Effects of EC, EGC, EGCG and ECG on force of contraction in fura-2/AM unloaded left atrial muscle preparations:

In the first experiment, the effect of the catechins on the force of contraction was examined using left atrial muscle preparations without fura-2/AM loading. EC, EGC, EGCG or ECG was added to the incubation solution after an equilibration period of 90 to 120 min. The force of contraction at the beginning of the experiment (before drug addition) was 10.8 ± 0.7 mN ($n=20$).

Under the experimental conditions employed in this study, EC and EGC had no effect on the force of contraction (Fig. 2), but EGCG and ECG increased the force of contraction in a dose-dependent manner. The maximum force of contraction by EGCG and ECG was $234.6 \pm 31.0\%$ ($n=6$) and $277.4 \pm 25.5\%$ ($n=6$), respectively: the value before drug addition was set at 100% (Fig. 2). Despite there being no significant difference between the above two values, the ED_{50} calculated from the concentration-response curve by EGCG (39.7 ± 6.7 μ M, $n=6$) was significantly higher when compared to ECG (5.05 ± 0.32 μ M, $n=6$, Student's unpaired t -test, $P<0.01$). No marked influence on the resting tension by EGCG or ECG was observed. Thus, the influence on the force of contraction of the left atrial muscle preparations by the catechins varied.

Effects of EGCG and ECG on the force of contraction and $[Ca^{2+}]_i$ in fura-2/AM loaded left atrial muscle preparations:

It was reported that the increase in force of contraction by EGCG at a low concentration (10 μ M) accompanied an increase in the amplitude of $[Ca^{2+}]_i$, but it was not observed at a high concentration (100 μ M) [6], even when a marked

positive inotropic effect was observed. In addition, ECG did not increase $[Ca^{2+}]_i$ [8]. To clarify these issues, based on experimental results using left atrial muscle preparations without fura-2 loading (Fig. 2), we selected 20 μM as a low concentration of EGCG and 100 and 300 μM were used for high concentrations. Meanwhile, 5 and 50 μM of ECG were selected for low and high concentrations, respectively. We then examined the relationship between the increase in the force of contraction and changes in the amplitude of $[Ca^{2+}]_i$. Under the conditions without drug influence, the force of contraction of the fura-2/AM loaded left atrial muscle preparations was 7.88 ± 0.84 mN ($n=9$). The amplitude of $[Ca^{2+}]_i$ was 1.13 ± 0.04 ($n=9$). The time taken to reach the peak of the twitch tension and $[Ca^{2+}]_i$ were 86.8 ± 1.7 msec and 82.8 ± 1.9 msec ($n=9$), respectively.

Addition of 20 μM EGCG to the perfused solution increased the force of contraction to its peak in about 20 min (Fig. 3). At the maximum force of contraction, the force was reached to 213.8 $\pm 9.8\%$ of control values: the value before drug addition was set at 100% (Figs. 3 and 4). EGCG at high concentrations (100 and 300 μM) further increased the force of contraction. The time taken to reach the peak of the twitch tension was prolonged by EGCG in a dose-dependent manner (Table 1).

EGCG, 20 μM , slightly increased the amplitude of $[Ca^{2+}]_i$, while EGCG at 100 and 300 μM significantly increased it (Figs. 3 and 4). The time taken to reach the peak of $[Ca^{2+}]_i$ was also prolonged (Table 1). Addition of ECG at 5 or 50 μM also increased the force of contraction and the amplitude of $[Ca^{2+}]_i$ in a dose-dependent manner (Fig. 4). The time taken to reach the peak of the twitch tension and $[Ca^{2+}]_i$ were also significantly prolonged (Table 1).

Figure 5 shows the increases in the force of contraction by EGCG (20, 100 and 300 μM), ECG (5 and 50 μM) and $CaCl_2$ (1.8, 3.0, and 4.8 mM) which were plotted on the longitudinal axis (the value before drug addition was set at 100%), while the increases in the amplitude of $[Ca^{2+}]_i$ were plotted on the horizontal axis. Between the two values, there was a positive correlation for EGCG ($r=0.70$, $P<0.004$), ECG ($r=0.67$, $P<0.04$), and $CaCl_2$ ($r=0.78$, $P<0.001$). When the increase in the force of contraction was adopted for the objective variable (y) and the increase in the amplitude of $[Ca^{2+}]_i$ for the explanatory variable (x), the regression lines of EGCG, ECG, and $CaCl_2$ were calculated as $y=10.4x - 876$, $y=10.1x - 855$, and $y=10.7x - 923$, respectively. The regression coefficients of the regression lines for EGCG and ECG (EGCG: $t=0.088$, $P<0.930$; ECG: $t=0.161$, $P<0.837$) showed no significant difference to the regression coefficient of $CaCl_2$.

DISCUSSION

The present study demonstrated that the influence on the force of contraction by the catechins varied, and that the increases in the force of contraction by tea catechins may be involved in the increase in amplitude of $[Ca^{2+}]_i$ in isolated guinea pig left atrial muscle preparations.

In the left atrial muscle preparations without fura-2/

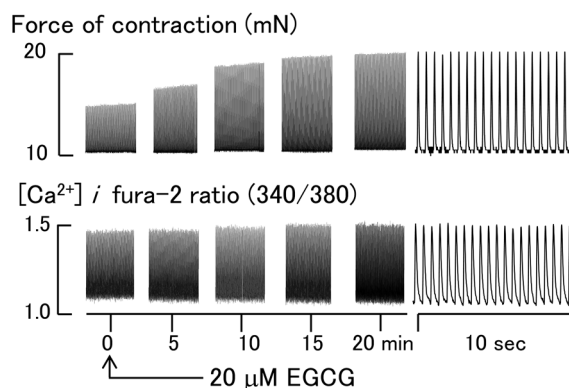


Fig. 3. Typical tracings of simultaneous measurements of force of contraction and $[Ca^{2+}]_i$ in guinea pig left atrial muscle preparation. Typical tracings are shown. Fura-2/AM loaded left atrial muscle preparations were horizontally suspended in a thin quartz glass bottom chamber containing 2 ml of the Krebs-Henseleit bicarbonate buffer solution, and were perfused with the buffer solution at a flow rate of 3 ml/min at 30°C under 2 Hz stimulation. The force of contraction and the $[Ca^{2+}]_i$ were measured simultaneously during the experimental period. Drugs were directly added to the perfusion solution. EGCG, 20 μM , was added at the point indicated by the arrow.

AM loading, EGC and EC had no influence on the force of contraction, but EGCG and ECG increased it in a dose-dependent manner. The maximum increase was not significantly different between EGCG and ECG, but the ED_{50} of EGCG, calculated from the concentration-response curve, was significantly larger than that of ECG. These results suggested that EGCG and ECG had comparable efficacy for the increase in force of contraction, but the affinity of receptor sites for ECG was higher than that for EGCG.

The catechins predominantly contained in green tea are EC, EGC, EGCG and ECG. The structural differences in these four compounds are the presence or absence of the hydroxyl group at the carbon 5'-position of the B ring and an esterified gallate moiety at the carbon 3'-position of the C ring [1, 5, 16] (Fig. 1). EC has neither gallate moiety nor hydroxyl group, while EGC has a hydroxyl group but no gallate moiety. ECG has a gallate moiety but no hydroxyl group, and EGCG has both the gallate moiety and hydroxyl group.

It has been considered that the gallate moiety at the 3'-position plays an important role in the biological activities of catechins [1, 5, 10, 16]. Indeed, in the Langendorff preparations obtained from rat hearts, Li *et al.* [8] reported that EC and EGC had almost no effect on the force of contraction, while EGCG and ECG increased the force. Moreover, they showed that ECG had a stronger effect than EGCG. Therefore, these results indicate that the gallate moiety was strongly involved in the increase in force of contraction by catechins. The hydroxyl group at the carbon 5'-position of the B ring, however, seems to decrease catechin affinity for the muscle receptor site.

In fura-2 loaded atrial muscle preparations, ECG also

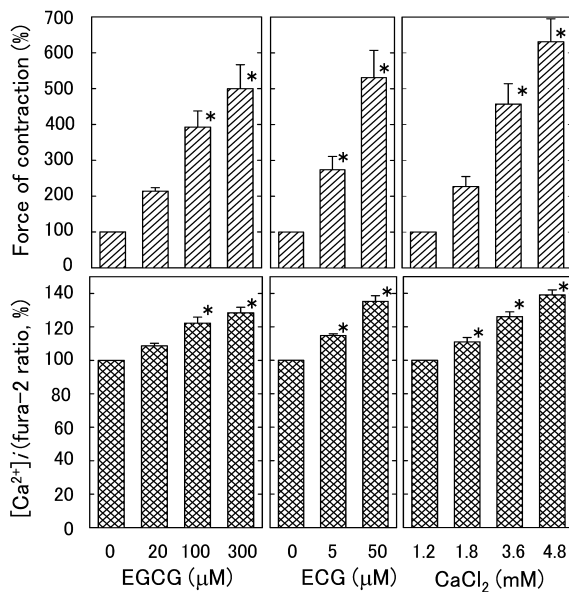


Fig. 4. Effects of EGCG, ECG and $CaCl_2$ on the force of contraction and the amplitude of $[Ca^{2+}]_i$ in guinea pig left atrial muscle preparations. See legend to Fig. 3. One preparation was examined for each concentration of EGCG (20, 100 and 300 μM) and ECG (5 and 50 μM). $CaCl_2$ concentrations were cumulatively increased from 1.2 to 1.8, 3.0 and 4.8 mM (When the peak at each concentration was observed, the next higher concentration was used. The interval was about 2–3 min). The force of contraction (upper panel): values just before addition of EGCG or ECG or an increase in $CaCl_2$ concentrations from 1.2 to 1.8 mM (control) were employed as 100%. $[Ca^{2+}]_i$ (lower panel): the range of 0 to 100% indicates the amplitude of $[Ca^{2+}]_i$. The value just before drug addition was set as the control (100%). Each value represents the mean of 5 to 7 experiments. Vertical lines indicate the standard error of the mean. An asterisk indicates a statistical significance compared with the control (Dunnett, $P < 0.05$).

caused a dose dependent increase in the force of contraction at a lower concentration when compared to the concentration which produced the increase in force of contraction by EGCG. This result also confirmed that the affinity of the receptor site, which relates to the increase in force of contraction, is higher for ECG compared with EGCG.

As the force of contraction increased, the amplitude of $[Ca^{2+}]_i$ also increased at low and high concentrations. The correlation between the increase in the force of contraction and the increase in the amplitude of $[Ca^{2+}]_i$ was strong for EGCG and ECG, and the calculated slopes of the regression line were comparable with those for $CaCl_2$. These results suggested that the increase in force of contraction by EGCG and ECG was closely related to the amplitude of $[Ca^{2+}]_i$. Lorenz *et al.* [9] reported that EGCG produced a dose-dependent increase in the fractional shortening of rat cardiac myocytes and intracellular systolic Ca^{2+} concentrations. Our results strongly supported their findings.

In addition, the experimental results observed in the present study and by Lorenz *et al.* [9] show that the increase in force of contraction and the amplitude of $[Ca^{2+}]_i$, were

Table 1. Effects of EGCG, ECG and $CaCl_2$ on time taken to reach the peak of the twitch tension and $[Ca^{2+}]_i$ in isolated fura-2/AM loaded left atrial muscle preparations

	Twitch tension (%)	$[Ca^{2+}]_i$ (fura-2 ratio, %)
EGCG		
20 μM	106.0 \pm 2.1 (4)	105.8 \pm 2.0 (4)
100 μM	108.0 \pm 1.6 (4)	107.0 \pm 1.7 (4)
300 μM	110.2 \pm 3.0 (5) ^a	110.0 \pm 2.4 (5) ^a
ECG		
5 μM	106.0 \pm 1.9 (5)	105.4 \pm 1.3 (5) ^a
50 μM	116.3 \pm 3.4 (5) ^a	113.2 \pm 2.0 (5) ^a

Values of the time taken to reach the peak of the twitch tension and $[Ca^{2+}]_i$ were estimated as the percentage of control values observed immediately before addition (set at 100 %) of EGCG and ECG. Values are the mean \pm SE. Numbers in parentheses represent numbers of experiments. a) Significantly different from control values observed immediately before drug addition (Dunnett, $P < 0.05$).

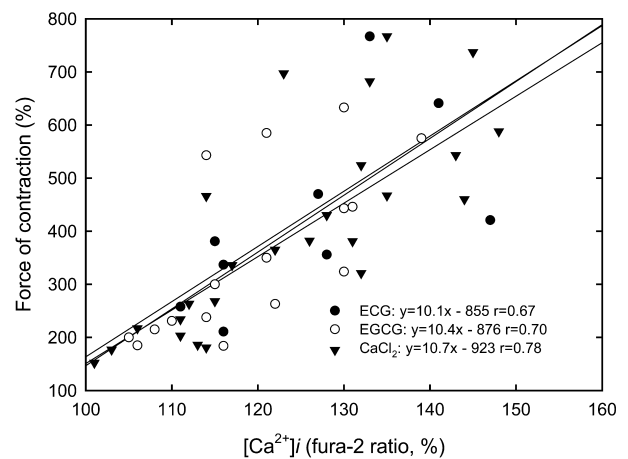


Fig. 5. Relationship between force of contraction and amplitude of $[Ca^{2+}]_i$ in guinea pig left atrial muscle preparations. See legend to Fig. 3. The increase in the force of contraction (%) by EGCG, ECG and $CaCl_2$ was plotted on the longitudinal axis, while the increase in the amplitude of $[Ca^{2+}]_i$ (%) was plotted on the horizontal axis. Field circle, ECG; open circle, EGCG; field triangle, $CaCl_2$; r, correlation coefficient; equation, regression formula.

produced by a similar concentration range of EGCG in both guinea pig atrial muscle preparations and rat cardiac myocytes. It is suggested that catechins may not produce species different actions on the force of contraction and $[Ca^{2+}]_i$.

Tadano *et al.* [13, 14] reported that EGCG and ECG decreased the Ca^{2+} sensitivity of the contractile protein in skinned fiber preparations of rabbit ventricular muscles. They proposed these catechins might be effective in treating hypertrophic cardiomyopathy. However, when the Ca^{2+} sensitivity of the contractile protein was decreased in cardiac muscle cells, a decrease in the force of contraction generally occurs [15], however, we observed an increase in the force of contraction.

In addition, the correlation between the increase in force of contraction and amplitude of $[Ca^{2+}]_i$ induced by EGCG

and ECG would not be comparable with the effects induced by CaCl_2 : a marked increase in amplitude of $[\text{Ca}^{2+}]_i$ by EGCG or ECG that would overwhelm the decreased Ca^{2+} sensitivity would be required. Our results indicated that Ca^{2+} sensitivity was not affected by EGCG and ECG in myocardial preparations with intact cell membranes.

Recently, it has been reported that EGCG suppressed activities of the Na^+ , K^+ -ATPase [12] and the Na^+ , Ca^{2+} exchange system [9]. The dose-dependent suppression of Na^+ , K^+ -ATPase activity revealed that ECG had the lowest IC_{50} value, followed by EGCG, which follows the same trend as the ED_{50} values calculated in the present study. In addition, values for EC and EGC could not be calculated [12]. It was also proposed that the rate of extrusion of $[\text{Ca}^{2+}]_i$, via a Na^+ , Ca^{2+} exchanger, plays an important role in heart muscle relaxation [2]. Inhibition of this exchanger, and an ensuing delay in the Ca^{2+} extrusion from cells, causes the prolongation of the time taken to the peak of twitch tension and an increase in force of contraction. In the present study, EGCG and ECG prolonged the time taken to reach the peak of the twitch tension and of $[\text{Ca}^{2+}]_i$. Furthermore, it has been reported that EGCG is highly water soluble [5]. Therefore, it was presumed that the increase in force of contraction was mediated by the binding of EGCG and ECG to receptor sites located at the surface of or within the cell membrane and they did not require permeability into the cell.

In summary, in left atrial muscle preparations without fura-2/AM loading repeatedly stimulated at 2 Hz, EGCG and ECG increased the force of contraction in a dose-dependent manner. Their efficacy was comparable, but the affinity for ECG was higher. Also, in the fura-2 loaded left atrial muscle preparations, EGCG and ECG increased the force of contraction in a dose-dependent manner and increased the amplitude of $[\text{Ca}^{2+}]_i$. There was a strong positive correlation between the increase in the force of contraction and the amplitude of $[\text{Ca}^{2+}]_i$; in addition, the calculated slopes of the regression lines were comparable with those calculated for CaCl_2 . These results suggested that the increase in force of contraction by EGCG and ECG was closely related with the increase in amplitude of $[\text{Ca}^{2+}]_i$ produced by CaCl_2 .

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