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Mechanisms underlying the vasorelaxation of human internal mammary artery induced by (-)-epicatechin

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ABSTRACT

Evidences have suggested that flavanol compound (-)-epicatechin is associated with reduced risk of cardiovascular diseases. One of the mechanisms of its cardioprotective effect is vasodilation. However, the exact mechanisms by which (-)-epicatechin causes vasodilation are not yet clearly defined. The aims of the present study were to investigate relaxant effect of flavanol (-)-epicatechin on the isolated human internal mammary artery (HIMA) and to determine the mechanisms underlying its vasorelaxation. Our results showed that (-)-epicatechin induced a concentration-dependent relaxation of HIMA rings pre-contracted by phenylephrine. Among the K^+ channel blockers, 4-aminopyridine (4-AP) and margatoxin, blockers of voltage-gated K^+ (K_V) channels, and glibenclamide, a selective ATP-sensitive K^+ (K_{ATP}) channels blocker, partly inhibited the (-)-epicatechin-induced relaxation of HIMA, while iberiotoxin, a most selective blocker of large conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), almost completely inhibited the relaxation. In rings pre-contracted by 80 mM K^+, (-)-epicatechin induced partial relaxation of HIMA, whereas in Ca^{2+}-free medium, (-)-epicatechin completely relaxed HIMA rings pre-contracted by phenylephrine and caffeine. Finally, thapsigargin, a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor, slightly antagonized (-)-epicatechin-induced relaxation of HIMA pre-contracted by phenylephrine. These results suggest that (-)-epicatechin induces strong endothelium-independent relaxation of HIMA pre-contracted by phenylephrine whilst 4-AP- and margatoxin-sensitive K_V channels, as well as BK_{Ca} and K_{ATP} channels, located in vascular smooth muscle, mediate this relaxation. In addition, it seems that (-)-epicatechin could inhibit influx of extracellular Ca^{2+}, interfere with intracellular Ca^{2+} release and re-uptake by the sarcoplasmic reticulum.

1. Introduction

During the last 20 years around 200 vasodilator compounds derived from plants, like resveratrol, quercetin, procyanidins, have been identified as well as their possible mechanism(s) of action (Aldini et al., 2003; Novakovic et al., 2006; Pérez-Vizcaíno et al., 2002). Despite the number and the structural diversity, it is clear that most compounds with vasodilator activity are alkaloids, terpenoids or flavonoids. The main mechanisms involved in the action of the most plant-derived vasodilators are: the activation of NO/cGMP and PGI_2/cAMP pathways, the activation of K^+ channels and blockade of voltage-dependent Ca^{2+} channels. Furthermore, it is demonstrated that about 40% of these vasodilator compounds have more than one mechanism of action (Luna-Vázquez et al., 2013).

(-)-Epicatechin is a natural compound present in high concentrations in grapes, cocoa, green tea, apples, and many other fruits and vegetables (Vazquez-Prieto et al., 2012). Along with catechin and their oligomers, the procyanidins, it belongs to flavanols, a major group of flavonoids in human diet (Heiss et al., 2010). In the recent years, the epidemiological studies suggest that high dietary intake of flavanols is associated with reduced risk of cardiovascular diseases (Buijsse et al., 2006; Deka and Vita, 2011; Ding et al., 2006; Mukamal et al., 2002). (-)-Epicatechin seems to

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be a major bioactive constituent of cocoa and other flavanol-rich foods and beverages (Gómez-Guzmán et al., 2012). Many studies have focused on the protective effects of pure (-)-epicatechin in the cardiovascular system (Jiménez et al., 2012). For example, it has been shown that (-)-epicatechin improves vascular function in humans, as evaluated by measuring flow mediated dilation, and that its content correlates with blood pressure lowering effect of flavanol-rich food (Ellinger et al., 2012; Schroeter et al., 2006). In addition, experiments in animal models have also demonstrated that (-)-epicatechin prevents and reverses hypertension, improves endothelial dysfunction, reduces the vascular oxidative stress and proinflammatory status, early events involved in development of atherosclerosis and insulin resistance (Galleano et al., 2013; Gómez-Guzmán et al., 2012, 2011; Litterio et al., 2012; Vazquez-Prieto et al., 2012).

In vitro, (-)-epicatechin induces endothelium-independent and endothelium-dependent nitric oxide (NO)-mediated vasodilation in resistance and conduit arteries (Aggio et al., 2013; Huang et al., 1999, 1998). Huang et al. (1999) found that the vasorelaxing effects of (-)-epicatechin in rat mesenteric arteries were partially attenuated by the nitric oxide synthase inhibitor L-NAME, as well as by removal of the endothelium. Further, (-)-epicatechin-induced relaxation in endothelium-intact tissues may be also mediated by NO-dependent activation of iberiotoxin-sensitive K^+ channels (Huang et al., 1999). On the other hand, it has been shown that inhibition of Ca^{2+} influx via voltage-gated Ca^{2+} channels could mediate the endothelium-independent relaxation induced by (-)-epicatechin (Huang et al., 1998).

However, the exact mechanisms by which flavanol compound (-)-epicatechin causes vasodilation are not yet clearly defined. Thus, the principal aims of the present study were to investigate vasorelaxant effect of (-)-epicatechin on the isolated human internal mammary artery (HIMA) and its underlying mechanisms, focusing on the role of different K^+ channels.

2. Materials and methods

2.1. Tissue preparation

Samples obtained from discarded segments of HIMA ($n=73$) from patients undergoing coronary artery bypass grafting were immediately placed in cold (4 °C) Krebs-Ringerbicarbonate solution and transported to the laboratory. There were 49 male patients (mean age ± standard error of mean (S.E.M.); 61 ± 5 years) who gave their informed consent for the usage of remaining tissue. The study protocol was approved by the Ethics Committee of Institute for Cardiovascular Diseases "Dedinje", and investigation conforms with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

The HIMA segments were dissected free of the fat and connective tissue and cut into 3 mm rings. One or two rings were obtained from each vessel segment. In some experiments, the endothelium was removed mechanically by gently rubbing the lumen with a stainless steel wire. Removal of endothelium was verified by the loss of relaxation induced by acetylcholine (1 μM).

The rings were placed in a 10 ml organ bath filled with Krebs-Ringerbicarbonate solution, maintained at 37 °C and continuously gassed with a mixture of 95% O_2 and 5% CO_2. During 90 min of equilibration under a resting tension of 2 g, rings were rinsed with fresh solution every 10 min.

2.2. Experimental protocol

After equilibration period, the HIMA rings were contracted with phenylephrine (10 μM). When a stable contraction was achieved (approximately 15–20 min), the cumulative concentration–response curves to (-)-epicatechin (0.1 μM to 500 μM) were established. Increasing concentrations of (-)-epicatechin were added after the relaxation evoked by the previous concentration reached its plateau or after 20 min if no response was obtained. Experiments were performed in both endothelium-intact and endothelium-free arteries to determine whether the relaxant effect of (-)-epicatechin is endothelium-dependent. A time-matched control was obtained using an equivalent volume of the solvent as that used for dissolving (-)-epicatechin.

As the preliminary data showed that endothelium denudation did not significantly reduce relaxation to (-)-epicatechin, the subsequent experiments were performed on endothelium-denuded rings.

2.3. Role of K^+ channels in (-)-epicatechin-induced relaxation

In order to evaluate the role of K^+ channels in (-)-epicatechin-induced relaxation, the effects of different K^+ channel blockers were examined on endothelium-denuded rings. Therefore, the following protocol was used: 1) contraction with phenylephrine and concentration–response curve to (-)-epicatechin followed by three washes, addition of the different K^+ channel blocker, and a 20-min equilibration period and 2) contraction with phenylephrine and the concentration–response curve to (-)-epicatechin. Rings with no added blocking drug were taken as control.

2.4. Role of Ca^{2+} in (-)-epicatechin-induced relaxation

Effects of (-)-epicatechin were examined on 80 mM K^+-contracted rings. K^+-rich Krebs-Ringer bicarbonate solution was prepared by replacing NaCl with an equivalent amount of KCl to maintain physiological osmolality. At the plateau of the high K^+ contraction, increasing concentrations of (-)-epicatechin were added. In some experiments, rings were pretreated with nifedipine, specific L-type Ca^{2+} channel blocker, for 20 min before contraction with 80 mM K^+.

In separate set of experiments, rings were incubated in Ca^{2+}-free Krebs-Ringer bicarbonate solution (without $CaCl_2$) before contraction with phenylephrine or caffeine (25 mM) and construction of the concentration–response curve to (-)-epicatechin.

Finally, some rings were incubated with thapsigargin, a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor, for 40 min before contraction with phenylephrine followed by relaxation to cumulative addition of (-)-epicatechin.

2.5. Treatment of data and statistics

The relaxation produced by each concentration of (-)-epicatechin was measured and expressed as a percentage of the maximum possible relaxation (i.e., relaxation back to the baseline tension). The concentration of (-)-epicatechin producing 50% of the maximum response (EC_{50}) was calculated from each concentration–relaxation curve using a non-linear least squares fit of individual experimental data and presented as pD_2 ($pD_2 = -\log EC_{50}$).

The results are expressed as the mean ± standard deviation (S.D.). The value of n indicates the number of experiments. Significant difference between means of different groups was determined by unpaired Student's t-test, and a P value < 0.05 was considered statistically significant. All calculations were performed using the SPSS statistical software (version 10.0; International Business Machines Corp, Armonk, NY).