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The Janus face of chlorogenic acid on vascular reactivity: A study on rat isolated vessels

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ABSTRACT

Background: Chlorogenic acid (CGA), the main polyphenol contained in coffee, is a major contributor to dietary polyphenol intake. Few studies reported its anti-hypertensive properties but the mechanisms are still indefinite.
Purpose: The present study assessed the direct effect of CGA in endothelium denuded or intact aortic rings from male Wistar rats and the mechanisms involved.
Methods/Results: CGA induced a direct endothelium-dependent relaxation that was significantly reduced by L-NAME (10^{-4} M), indomethacin (10^{-5} M) and combination of apamin (10^{-7} M) and charybdotoxin (10^{-7} M). Incubation of rings with CGA induced a dual effect on agonist-induced vasorelaxation. At 10^{-6} M, it enhanced the relaxant effects of acetylcholine and reduced the contracting effects of phenylephrine due to increased basal and stimulated NOS activity, respectively. At 10^{-4} M, CGA blunted acetylcholine and bradykinin-induced vasorelaxation, reduced phenylephrine-induced vasoconstriction but did not change the response to sodium nitroprusside, a NO-donor.
Conclusion: In summary, CGA induces a direct endothelium-dependent vasodilation by increasing NOS, COX and EDHF signalling pathways. However, this new pharmacological action that can explain some positive effects of CGA in case of hypertension has to be modulated at the light of its deleterious impact on vascular relaxation at high concentrations and incite to be cautious when using high doses of CGA in clinical studies.

Introduction

Chlorogenic acids are a family of esters formed between quinic and cinnamic acids, which are an important group of phenolic plant secondary metabolites, produced by certain plant species and present in the human diet (Clifford, 1999). The most common individual chlorogenic acid is 5-O-caffeoylquinic acid, which is still often called chlorogenic acid (CGA), with intakes reaching 1000 mg/day or more (Clifford, 1999). The primary dietary source of CGA is green and processed coffee beans (Liang and Kitts, 2016). However, besides coffee, CGA is also present in high amounts in various *fruits* such as pears, apples, plums, cherries, peach, kiwis, and *vegetables*, such as potatoes, carrot, tomato, sweet potato, Chicorium endivia, eggplants (Upadhyay and Mohan Rao, 2013; Liang and Kitts, 2016). It is assumed that CGA is mainly responsible for the health benefits suggested by epidemiologhical studies of consuming coffee, tea, fruit juice, and vegetable juice (Liang and Kitts, 2016). CGA is also found as a significant component in certain commonly used herbs in traditional medicines such as *Lonicera japonica* flowers (*jinyinhua*) and Eucommia bark (Upadhyay and Mohan Rao, 2013), gardenia fruit (Lee et al., 2014), artichoke leaves (Speroni et al., 2003), chrysanthemum (Wang et al., 2008), Artemisia annua (De Magalhaes et al., 2012), oilseeds (Pedrosa et al., 2000) and kuding tea (Wang et al., 2008).

CGA has attracted considerable attention in view of its beneficial effects on health. Previous experimental studies reported that it displayed antioxidant (Upadhyay and Mohan Rao, 2013), antibacterial (Karunanidhi et al., 2013), anti-inflammatory (Liang and Kitts, 2016), antiplatelet and antithrombotic (Fuentes et al., 2014) activities. Clinical studies showed that a diet rich in CGA prevented various diseases associated with oxidative stress such as cancer, cardiovascular, aging and neurodegenerative diseases

Abbreviations: cga, chlorogenic acid; L-name, nω-nitro-l-arginine methyl ester; Ach, acetylcholine; No, nitric oxide; NOS, NO synthase; COX, cyclooxygenase; EDHF, endothelium-derived hyperpolarizing factor.

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(Manach et al., 2004; Fujioka and Shibamoto, 2008). In recent years, a few studies on animal models of hypertension highlighted the potential antihypertensive effects of CGA intake (Suziki et al., 2002; Suziki et al., 2006; Zhao et al., 2012). These data were confirmed in a clinical study demonstrating the blood pressure lowering effect of CGA diet in hypertensive patients (Watanabe et al., 2006). Overall results suggested that the consumption of CGA might provide an effective approach for treatment of high blood pressure. However, the mechanisms involved in the antihypertensive effects of CGA remains unraveled and whether the hypotensive action of CGA relies on direct or indirect vascular mechanism is indefinite.

In the present study, we hypothesized that CGA has direct effects on the vasculature. Experiments were conducted on the widely-used model of isolated aortic rings in rats. We investigated the direct effect of CGA on aortic rings with or without endothelium and determined the mechanisms involved. We also determined whether CGA impacts the effect of physiologically-relevant modulators of vascular motricity.

Materials and methods

Chemicals

Chlorogenic acid hemihydrate (PubChem CID: 24981351); Phenylephrine hydrochloride (PubChem CID: 5284443); Acetylcholine chloride (PubChem CID: 6060); Nomega-nitro-L-arginine methyl ester (PubChem CID: 135193); Apamin (PubChem CID: 16218850); Charybdotoxin (PubChem CID: 56842037); Indometacin (PubChem CID: 3715); Bradykinin (PubChem CID: 439201); Sodium nitroprusside anhydrous (PubChem CID: 11963579).

Animals

Twenty three male Wistar rats (7–9 weeks old) were purchased from Janvier (Le Genest Saint Isle, France). Animals were kept under a 12 h – 12 h light: dark cycle and allowed free access to food and water. The experimental procedures were approved by the local committee for ethics in animal experimentation n° 2012/001-CD of Franche-Comté University (Besançon, France), and complied with the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication No. 85–23, revised 2011) and with the '*Animal Research: Reporting In Vivo Experiments*' ARRIVE guidelines.

Vascular reactivity

After anesthesia with sodium pentobarbital (60 mg/kg, ip) the thoracic aorta was excised, cleaned of connective tissue, and cut into rings of approximately 2 mm in length. Rings were suspended in Krebs solution (mmol/l: NaCl 118, KCl 4.65, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 24.9, $MgSO_4$ 1.18, glucose 12, pH 7.4), maintained at 37 °C and continuously aerated with 95% O_2, 5% CO_2, for isometric tension recording in organs chambers, as previously described (Demougeot et al., 2005). In some rings, endothelium was mechanically removed. The completeness of endothelial denudation was confirmed by the absence of relaxation to the endothelium-dependent agonist acetylcholine (ACh, 10^{-6} mol/l). After a 90 min-equilibration period under a resting tension of 2 g.

Experimental procedures

Direct relaxant effects of chlorogenic acid on aortic rings and mechanisms involved

Chlorogenic acid hemihydrate (CGA), (purity 97%, B21962, lot 10142590) was obtained from Alfa Aesar, A Johnson Matthey Company (Germany). To evaluate the vasorelaxant effects of CGA on aortic rings, experiments were performed using rings with intact endothelium and denuded rings. Rings were preconstricted with phenylephrine (PE, 10^{-6} mol/l). Once the plateau was reached, CGA was added in the bath in a cumulative manner (10^{-10}– 10^{-4} mol/l). To investigate the mechanisms involved in CGA-induced relaxation, the contribution of nitric oxide synthase (NOS), cyclooxygenase (COX) and the endothelium-derived hyperpolarizing factor (EDHF) was assessed in endothelium-intact aortic rings preincubated with the NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME, 10^{-4} mol/l), the COX inhibitor indomethacin (10^{-5} mol/l) or the calcium-dependent potassium channels inhibitors apamin and charybdotoxin (10^{-7} mol/l) for 60 min. Cumulative concentrations of CGA (10^{-10} – 10^{-4} mol/l) were then applied during the sustained phase of PE (10^{-6} mol/l)-induced contraction.

Effects of CGA on Ach-induced relaxation

To determine whether CGA induces changes in the response of aortic rings to relevant agonist, the effects of ACh (10^{-11}–10^{-4} mol/l) were evaluated in rings constricted with PE (10^{-6} mol/l) in the absence or the presence (30 min incubation) of CGA at 2 concentrations ("low" 10^{-6} mol/l and "high" 10^{-4} mol/l).

Role of NOS pathway in the CGA effects at low concentration

In order to study whether the impact of low CGA on ACh-induced relaxation involves the NOS pathway, rings were constricted with PE (10^{-6} mol/l) and the cumulative effects of ACh (10^{-11}–10^{-4} mol/l) were studied in absence or in the presence of either L- NAME (10^{-5} mol/l) or L- NAME (10^{-5} mol/l) plus CGA (10^{-6} mol/l). To determine whether the low CGA might also affect the basal NOS activity, PE (10^{-11} to 10^{-4} mol/l) was added cumulatively to the organ bath in the presence or not of CGA (10^{-6} mol/l) or CGA plus L- NAME (10^{-5} mol/l).

Mechanisms involved in the effects of high CGA concentration on agonist-induced vasoreactivity

First, to determine whether the effects of high CGA on agonist-induced relaxation are dependent on the receptor activated, we investigated the effects of bradykinin (BK, 10^{-9}–10^{-4} mol/l) in rings preconstricted with PE (10^{-7} mol/l) pre-incubated or not with CGA (10^{-4} mol/l). Second, to determine whether the deleterious effect of CGA on agonist-induced relaxation was secondary to an enhancement of the response to PE, PE (10^{-11} to 10^{-4} mol/l) was added cumulatively to the organ bath in the presence or not of CGA (10^{-4} mol/l). Third, to assess the effect of high CGA on guanylate cyclase activity in vascular smooth muscle cells, the relaxant response to the NO donor sodium nitroprussiate (SNP, 10^{-11} to 10^{-4} mol/l) was measured in preconstricted rings (PE, 10^{-6} mol/l) in the presence or not of CGA (10^{-4} mol/l).

Data analysis and statistics

Values are presented as means ± SEM. Data were analyzed using GrapPad Prism version 5.03. Contractile responses to PE were expressed as the percentage of the maximum response to KCl 100 mM and relaxant responses to ACh and bradykinin were expressed as the percentage of relaxation of the contractile response to PE 10^{-6} mol/l. The EC_{50} and Emax values were calculated from individual concentration-responses curves by using the nonlinear regression analysis performed by GraphPad Prism software (version 5.03). Concentration-response curves were compared by two-way analysis of variance (ANOVA) for repeated measures. When necessary, to better understand the effect of inhibitors, the results were expressed as the area under the curve (AUCs) calculated from the individual concentration-response curves. Comparison between two values was assessed by unpaired Student *t* test or Mann–Whitney test when data were not normally distributed. $P < 0.05$ was considered statistically significant.