Iranian Journal of Pharmaceutical Research (2006) 3: 199-202
Received: October 2004
Accepted: November 2005

Original Article

The Involvement of L-Type Voltage-Operated Calcium Channels in the Vascular Effect of Quercetin in Male Rats

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Abstract

In this study, the possible involvement of L-type voltage-operated calcium channels in the vasorelaxant effect of the flavonoid quercetin was investigated, using the isolated aortic rings from normal male rats. Addition of quercetin (0.1 μM-1 mM) caused a significant dose-dependent relaxation of noradrenaline (NA)- and KCl-preconstricted rings and nifedipine attenuated this response, especially for noradrenaline ($P<0.05$). It is concluded that the flavonoid quercetin produces a potent relaxation of the rat aorta and blockade of voltage-operated calcium channels could attenuate this effect.

Keywords: Quercetin; Aorta; Calcium channel; Rat.

Introduction

Flavonoids comprise a large group of secondary metabolites widely distributed throughout the plant kingdom, including food plants. Epidemiological studies have shown an inverse association between dietary flavonoid intake and mortality from coronary heart disease (1). Among dietary flavonoids, quercetin is by far the most abundant, representing approximately 60% of the total intake (2). Regarding this flavonoid, a very wide range of biological actions has been reported (3). In fact, the latter drug exerts antioxidant (4), antiaggregant (5), and vasodilator effects, which may help to explain its' cardiovascular protective effects (6). In addition, it has been recently reported that quercetin exerts antihypertensive effects and reduces left ventricular hypertrophy, endothelial dysfunction, and the plasma and hepatic oxidative status in spontaneously hypertensive rats (7). A limitation for the understanding and relevance of these findings is the existence of the scarce and conflicting data on how the flavonoids modify the contractility of the vascular system. The vasodilatory effect of quercetin and its' metabolites has been established before, but the role of calcium channels in mediation of this effect has been poorly understood (8, 9). Therefore, this experimental study was conducted to evaluate the role of voltage-gated calcium channels in the vasorelaxant effect of quercetin in male normal rats.

Experimental

Animals

Male albino Wistar rats weighing 235-275 g (8-10 weeks old) were housed in an air-conditioned colony room (21± 2°C with a humidity of 30-40%) and supplied with standard pellet diet and tap water ad libitum. Procedures involving animals and their care were conducted

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in conformity with the institutional guidelines of Shahed University (Tehran, Iran) and in accordance to the NIH guidelines for the care and use of laboratory animals.

Experimental procedure

The rats (n=20) were anesthetized with diethyl ether, decapitated, and then through opening the abdomen, the descending thoracic aorta was carefully excised and placed in cold physiological saline solution (PSS) containing (mM): NaCl 118, KCl 4.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 27.2, and $CaCl_2$ 1.8. Thereafter, the aorta was cleaned of excess connective tissue and fat and cut into rings of approximately 4 mm in length. Aortic rings were suspended between two triangular-shaped wires. One wire was attached to a fixed tissue support in a 50 ml isolated tissue bath containing PSS (pH 7.4) maintained at 37°C and continuously aerated with a mixture of 5% CO_2 and 95% O_2. The other end of each wire attached by a cotton thread to a F60 isometric force transducer (Narco Biosystems, USA) coupled to a signal amplifier and connected to a Pentium-III (IBM-compatible) computer via an A/D interface. Recording and analysis of data was performed using the Physiograph I software (Behineh Arman Co., Tehran). In all experiments, special care was taken to avoid damaging the luminal surface of endothelium. The rings were allowed to equilibrate for 90 min under a resting tension of 1.5 g, before the start of experiments. This had been shown in preliminary experiments to be the optimal resting tension. During the equilibration, the rings were washed every 30 min. For examining the endothelial integrity, rings pre-constricted with noradrenaline (NA, 1μM) were exposed to a single addition of acetylcholine (ACh, 10 μM). Only those endothelium-intact rings exhibiting more than 50% relaxation in response to ACh were used for further experimentation. In all experiments, after the addition of each dose, a plateau response was obtained before the addition of a subsequent dose. Furthermore, all experiments for NA were performed in the presence of 1 μM timolol, 1 μM imipramine, and 1 μM prednisolone to eliminate the effects of β-adrenoceptors, neuronal uptake, and extraneuronal uptake respectively.

After an initial equilibration, the aortic rings were allowed to achieve maximal tension by exposure to a high K^+ solution (80 mM), which was prepared by replacing the NaCl concentration of PSS with an equimolar concentration of KCl. Then, the relaxant responses to different concentrations of quercetin (0.1 μM-1 mM) were recorded. After PSS rinsing (3 times within a period of 30 min), the rings were constricted with NA (1μM) and again the relaxant responses to the same concentrations of quercetin were recorded. The quercetin-evoked vasorelaxation was expressed as a percentage of relaxation and the IC_{50} (the concentration which produced a 50% maximal relaxation) was determined from the concentration-response curves. The involved mechanisms underlying the vasorelaxant action of quercetin was examined by pretreatment of some aortic rings (n=15) with nifedipine, 5 min before the addition of vasoconstrictors and quercetin.

Drugs and chemicals

Noradrenaline bitartrate, acetylcholine-HCl, quercetin, and streptozotocin were purchased from Sigma Chemical (St. Louis, Mo., USA). Prednisolone, imipramine, and timolol were obtained from Darupakhsh and Sinadarou pharmaceutical companies (Tehran, Iran) as a gift. All other chemicals were purchased from Merck (Germany). The flavonoid quercetin and calcium channel blocker nifedipine were dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO was less than 0.08%, which was shown to be devoid of any observable effects on the smooth muscle tone. Meanwhile, cumulative addition of this vehicle (DMSO) had no significant effect (at most 1.5-4% relaxation at the highest concentration of DMSO) on the thoracic aorta. Further dilutions of the drugs were made in PSS.

Data and statistical analysis

All the data abtained are presented as mean ± S.E.M. and analyzed by the student's t-test, with a significant level of $P<0.05$.

Results and Discussion

Noradrenaline (1μM) induced a sustained contraction of the rat aorta, with a peak tension

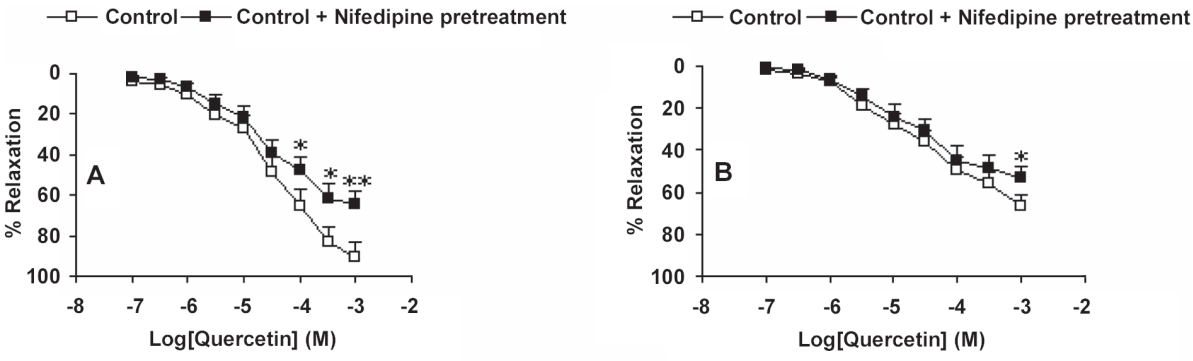

Figure 1. Vasorelaxant effect of quercetin against NA (A) and high K^+ (B)-induced contractions in aortic rings and the effect of nifedipine pretreatment * P<0.05, ** P<0.01

of 758.23 ± 36.7 mg. Addition of quercetin to the aortic rings induced a dose-dependent relaxation of the rings pre-contracted with NA (Figure 1A). In this respect, quercetin-induced vasorelaxation of rings in the control group pretreated with nifedipine was significantly lower than that of the control group at concentrations higher than 100 μM (P<0.05 and P<0.01). The IC_{50} (in a logarithmic scale) was -4.42 ± 1.02 for the control group and –3.97 ± 1.16 for the control group pretreated with nifedipine. This difference did not reach to a significant level.

Addition of a high K^+ (80 mM)-containing PSS to the tissue bath induced a maximal tension of 317.2 ± 15.9 mg. Addition of quercetin to the aortic rings induced a dose-dependent relaxation of the rings pre-contracted with NA (Figure 1B). In this respect, quercetin-induced vasorelaxation of rings in the control group pretreated with nifedipine was significantly lower than that of the to control group, only at a concentration of 1 mM (P<0.05). Further more, the IC_{50} was also –3.82 ± 1.02 for the control group and –3.07 ± 1.16 for the control group pretreated with nifedipine. Their difference did not reach a significant level.

Vasodilator effects of flavonoids have already been reported in the literature and most of the flavonoids described to date exhibit a vasorelaxant relaxant effect (10). Previous reports have also shown the vasodilator effect of quercetin in isolated rat aorta (6). In this respect, many flavonoids, including quercetin, exert vasodilator effects probably through the release of endothelium-derived relaxing factors such as nitric oxide. It is well known that NO induces vascular smooth muscle relaxation through the activation of guanylyl cyclase, leading to the accumulation of cyclic GMP (11). In this regard, the results of previous studies have also shown that the quercetin-induced vasorelaxation in the aortic ring precontracted with noradrenaline is abolished by nitro L-arginine methyl ester (L-NAME), and quercetin produces significant increases in intracellular calcium level in the endothelial cells. This increase by quercetin has been abolished after removal of extracellular calcium. Therefore, quercetin through activating nitric oxide synthesis and release, by increasing intracellular calcium level in vascular endothelial cells, could exert its vasodilatory effect (12), and blockade of voltage-dependent calcium channels by nifedipine may attenuate the vasodilatory effect of this flavonoid, as observed in this study (12).

In summary, it can be concluded that the flavonoid quercetin produces a potent relaxation of the rat aorta and blockade of voltage-operated calcium channels could attenuate this effect.

Aknowledgement

This research study was financially supported by a grant from the Research Council of Shahed University (Tehran, Iran). The authors also gratefully appreciate Ms. Fariba Ansari for her excellent technical assistance.

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