Inhibition of Angiotensin-Converting Enzyme by Quercetin Alters the Vascular Response to Bradykinin and Angiotensin I

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Abstract
Quercetin, one of the most widely distributed flavonoids in the plant kingdom, inhibits various enzymes. This study examined its inhibitory effect on the angiotensin-converting enzyme activity through the cardiovascular response to bradykinin and angiotensin I. Quercetin pretreatment (88.7 μmol/kg p.o., 45 min; 14.7 μmol/kg i.v., 5 min) significantly potentiated the hypotensive effect of bradykinin (10 nmol/kg i.v.). This association was significantly attenuated by an antagonist of the B2 receptor. In addition, the hypertensive response to angiotensin I (0.1 nmol/kg i.v.) was significantly reduced by quercetin pretreatment using the same parameters as before. These results suggest an inhibitory effect of quercetin on the angiotensin-converting enzyme activity, similar to that of captopril. Quercetin was equally effective when given orally or intravenously.

Key Words
Angiotensin-converting enzyme inhibition · Quercetin · Blood pressure · Bradykinin · Angiotensin I

Introduction
Kinins are released from precursors, the kininogens, by kininogenases. Two subtypes of kinin receptors, B1 and B2, have been characterized based on their pharmacological responses to various bradykinin (BK) analogues. B1 kinin receptors are more sensitive to the kininase I metabolites des-Arg9-BK and des-Arg10-kallidin, whereas B2 kinin receptors are more abundant and have a greater affinity for BK and kallidin. Most of the effects of BK are mediated by B2 receptors, the stimulation of which leads, in turn, to the release of prostanoids and endothelium-derived relaxing factor. BK is thus involved in a variety of physiological mechanisms, including bronchoconstriction, plasma extravasation, release of prostaglandins/leukotrienes, smooth muscle contraction and relaxation, and nociception [1, 2].

Angiotensin (A) II regulates the blood pressure and is important in the pathogenesis of hypertension. The effects of AII on its target organs are mediated by plasma membrane receptors, of which AT1 receptors mediate most of the known functions of AII [3, 4].

Kininase II (EC 3.4.15.1) and neutral endopeptidase (NEP; EC 3.4.24.11), two dipeptidyl carboxypeptidases, are the major peptidases involved in the catabolism of...
were heparinized (100 IU/ml, 0.5 ml i.v.). Narco Biosystems physiograph. At the end of surgery the animals were exposed and intubated (polyethylene catheter, PE 240; Becton Dickinson) for direct measurements of blood pressure and for intravenous injection of drugs, respectively. The arterial pressure was monitored through an RP 1500 Narco Biosystems transducer connected to a four-channel luminal surface of the endothelial cell membrane as ectoenzymes [6] and show similarities in their active sites and in their respective mechanisms of action [7]. Both ACE and NEP can be inhibited by compounds containing a hydrophobic zinc-binding group [8].

Quercetin is one of the most widely distributed bioflavonoids in the plant kingdom. In recent years, it has been shown that these compounds affect a wide variety of biological systems in mammals, exhibiting antioxidant, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic effects [9, 10].

Flavonoids with free hydroxyl functions in positions 3, 3', 4', 5, and 7 have been shown to inhibit the activity of NEP [11], whilst the inhibition of ACE and aminopeptidase N (EC 3.4.11.2) does not appear to require the presence of these moieties. These results suggest that some of the pharmacological activities of flavonoids might be related to the inhibition of metallopeptidases.

In general, all inhibitory activities described are related to polyhydroxylated flavonoids. These interactions might be explained by the generation of chelate complexes with the zinc atom within the active site or, more likely, by a formation of hydrogen bridges between the inhibitor molecule and amino acids near to or at the active site, especially of their ortho-dihydroxyl groups in the case of quercetin.

The purpose of the present study was to examine the effects of quercetin on the ACE activity through the cardiovascular response to BK and AI in anesthetized rats.

**Materials and Methods**

The experiments were performed in male Wistar rats (weight 200–250 g) anesthetized with urethan (1.25 g/kg i.p.). The trachea was exposed and intubated (polyethylene catheter, PE 240; Becton Dickinson, Franklin Lakes, N.J., USA). As described previously [12], the carotid artery and the jugular vein were cannulated with heparinized polyethylene catheters (PE 50; Becton Dickinson) for direct measurements of blood pressure and for intravenous injection of drugs, respectively. The arterial pressure was monitored through an RP 1500 Narco Biosystems transducer connected to a four-channel Narco Biosystems physiograph. At the end of surgery the animals were heparinized (100 IU/ml, 0.5 ml i.v.).

The drugs used in this study were quercetin (fig. 1), icatibant [D-Arg-(Hypl, Thi3, D-Tic5, Oic6) bradykinin; Hoe 140] [13], BK, AI, captopril (fig. 1), heparin, and hippuryl-L-histidyl-L-leucine. Heparin was purchased from Cristália (São Paulo, Brazil). All other drugs were obtained from Sigma (St. Louis, Mo., USA). Quercetin was dissolved in 10% dimethylsulfoxide; BK, AI, and captopril were dissolved in 0.9% NaCl. The dose of BK was selected based upon the results of preliminary experiments in which animals were treated with captopril; similarly, the dose of quercetin was selected from preliminary experiments in which we compared the effects of 14.7, 29.4, and 88.7 μmol/kg (p.o.) on the vascular effects of BK and AI (results not shown).

Two groups of rats were pretreated with oral quercetin (88.7 μmol/kg) or oral captopril (100 nmol/kg), respectively, 45 min before the administration of BK (10 nmol/kg i.v.) and AI (0.1 nmol/kg i.v.). All other groups received an intravenous injection of quercetin (14.7 μmol/kg) or captopril (10 nmol/kg) 5 min before the administration of BK and AI at the same doses given above. After injections, the cannulae were flushed with 0.5 ml of saline solution.

Blood pressure recordings are given as mean values ± SEM of the mean arterial pressure (MAP).

A complementary in vitro enzyme assay was performed according to the method of Santos et al. [14]. In brief, blood samples were obtained by cardiac puncture, under light anesthesia, from two groups of animals previously treated with saline or quercetin (88.7 μmol/kg, p.o.). The plasmatic ACE activity was then determined by a fluorimetric assay in which 1 U of the ACE activity generates 1 μmol of L-histidyl-L-leucine per minute.

The statistical significance of differences between mean values was calculated using Student’s t test for nonpaired samples. p < 0.05 was regarded as significant.

**Results**

Figure 2 shows the effect of BK on the blood pressure in anesthetized rats following pretreatment with captopril or quercetin. Intravenous injection of BK (10 nmol/kg) had a marked hypotensive effect (18.0 ± 0.7 mm Hg). In rats treated with captopril (10 nmol/kg i.v.) 5 min before injection of BK or captopril (100 nmol/kg i.v.) 45 min before, there was a significant potentiation of the hypotensive response to BK to 27.6 ± 1.9 and 29.7 ± 1.8 mm Hg, respectively.

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**Fig. 1.** Chemical structures of quercetin and captopril.
Fig. 2. Increased hypotensive effect of BK after pretreatment with captopril or quercetin in anesthetized rats (oral route: captopril 100 nmol/kg, quercetin 88.7 μmol/kg; intravenous route: captopril 10 nmol/kg, quercetin 14.7 μmol/kg). Values represent the mean ± SEM of 4–6 experiments. * p < 0.05 (significant difference from control value).

Fig. 3. Reduction in MAP in anesthetized rats injected with AI alone and after pretreatment with captopril or quercetin via oral (captopril 100 nmol/kg; quercetin 88.7 μmol/kg) or intravenous (captopril 10 nmol/kg; quercetin 14.7 μmol/kg) route. Values represent the mean ± SEM of 4–6 experiments. * p < 0.05 (significant difference from control value).

Fig. 4. MAP in anesthetized rats injected with BK alone, following pretreatment with quercetin via oral route, and 5 min after intravenous administration of icatibant (10 nmol/kg) subsequent to pretreatment with quercetin. Values represent the mean ± SEM of 4–6 experiments. * p < 0.05 (significant difference from control value).

In a second set of experiments, we investigated the effect of AI on the blood pressure in anesthetized rats with and without captopril or quercetin pretreatment. The results of these experiments are shown in figure 3. Administration of AI (0.1 nmol/kg i.v.) caused a hypertensive response of 47.6 ± 2.6 mm Hg. Oral administration of captopril (100 nmol/kg) 45 min before the injection of AI or intravenous administration of captopril (10 nmol/kg) 5 min before significantly reduced the AI-induced hypertension by 16.3 ± 1.9 and 20.9 ± 2.5 mm Hg, respectively. Previous treatment with quercetin 45 min before (88.7 μmol/kg p.o.) or 5 min before (14.7 μmol/kg i.v.) injection of AI significantly reduced the hypertensive response to AI to 25.9 ± 2.1 and 28.1 ± 1.6 mm Hg, respectively.

In order to confirm that quercetin had no direct effect on the MAP, some animals were pretreated with the BK B2 receptor antagonist icatibant (Hoe 140; 10 nmol/kg i.v.) 5 min after oral administration of quercetin. Figure 4 shows that in these animals icatibant markedly inhibited the quercetin-enhanced BK hypotensive response, reducing the latter from 25.7 ± 1.9 to 3.98 ± 1.6 mm Hg.

In order to confirm that the effects of quercetin were due to ACE inhibition alone, the ACE activity was quantified in the plasma of two groups of rats treated with saline and quercetin, respectively. As shown in table 1, treatment of the animals with quercetin caused a significant inhibition of ACE.
The plasmatic ACE activity was then determined by a fluorimetric assay in which 1 U of the ACE activity generates 1 ìmol of L-histidyl-L-leucine per minute. The control and test ACE activities are expressed as the respective mean values ± SEM. The percentage inhibition was calculated with reference to the control ACE activity. *p < 0.05 (significant difference from control value).

| Table 1. Effect of quercetin on the plasma ACE activity |
|-----------------------------------|-----------------|------------------|
| Control ACE activity U/ml         | Test ACE activity U/ml | Inhibition % |
| 31.5 ± 1 (n = 6)                  | 21.6 ± 1* (n = 5)   | 31.32           |

The animals were treated with saline or quercetin (88.7 ìmol/kg). The plasmatic ACE activity was then determined by a fluorimetric assay in which 1 U of the ACE activity generates 1 ìmol of L-histidyl-L-leucine per minute. The control and test ACE activities are expressed as the respective mean values ± SEM. The percentage inhibition was calculated with reference to the control ACE activity. *p < 0.05 (significant difference from control value).

Discussion

The cardiovascular actions of ACE inhibitors are not mediated only by a reduction of AII, but also by the inhibition of the degradation of endogenous BK and related kinins. This is evidenced by the comparable effects of ACE inhibitors and exogenously added BK in different physiological and pathophysiological situations and by the observation that the specific B2 kinin receptor antagonist icatibant blocked the cardiovascular effects of ACE inhibitors as well as of BK in experimental models.

Continuous formation of BK occurs in the circulating blood at a level maintained very low by the various kininas present in plasma, tissues, and blood cells. Captopril also increases the local BK levels that affect vascular resistance. Thus, the local increase in BK levels might represent an additional factor in the lowering of the peripheral resistance [15].

In the present study, we showed that the flavonoid quercetin potentiates the hypotensive effect of BK and reduces the hypertensive effect of AI in anesthetized rats. In this study, pretreatment of rats with either captopril or quercetin caused a significant increase in the vasodepressor response to BK, independent of the route of administration.

It is well established that the major physiological role of ACE is the conversion of AI into AII [1]. Therefore, we investigated the effect of AI on blood pressure following pretreatment with the ACE inhibitor captopril or quercetin. The administration of AI caused a hypertensive response which was significantly reduced by the oral and the intravenous administration of captopril 45 and 5 min before, respectively. Similarly, previous treatment with oral and intravenous quercetin 45 and 5 min before, respectively, the injection of AI significantly reduced the hypertensive response to the latter. These results suggest an inhibitory effect of quercetin on the ACE activity which is comparable to that of captopril.

In the final series of experiments, we demonstrated that icatibant, a long-acting and highly specific BK B2 receptor antagonist [16], significantly attenuated the blood-pressure-lowering effect of the association between BK and quercetin in anesthetized rats. Icatibant administration had no significant effect on the baseline arterial blood pressure. However, this B2 receptor antagonist attenuated the blood pressure response to combined treatment with quercetin and BK. These results strongly suggest that the decreased catabolism of BK significantly contributed to the hypotensive response to quercetin in these experiments.

Flavonoids have been shown to inhibit a wide range of enzymes. In addition, flavonoids are effective antioxidants and inhibitors of lipid peroxidation; this is believed to result primarily from the radical-scavenging capacity of these polyphenols and secondarily from their action as chelators of metals that can catalyze the formation of radical species [17–21]. However, whilst the antioxidant properties of flavonoids are well documented, the precise contribution of metal chelation to flavonoid antioxidant activity is still unclear. Recently, it has been shown that in cell cultures exposed to tB-OOH, which exhibits potent, iron-dependent cytotoxic effects, cell death was significantly reduced by quercetin, thus suggesting an important role for metal chelation in the cytoprotective effects of this particular flavonoid [22]. Indeed, the authors of the latter study stated that the ability to chelate iron is the most prominent activity of quercetin.

The zinc metallopeptidases form a large group of enzymes which include ACE and NEP and are both membrane bound with similar topology and have similarities in their mechanisms of action [7], thereby permitting the inhibitory activities of both enzymes. The results demonstrate that some of this pharmacological activity of quercetin might be related to an inhibition of metallopeptidases responsible for splitting of peptides such as angiotensin and BK. Significant in the context of the present study, some authors suggest that a part of the in vitro activity of flavonoids may involve the generation of chelate complexes within the active center of ACE [23]. These findings have resulted, in part, from the introduction of an in vitro assay for detection of ACE inhibitors in plant extracts. This assay was subsequently used to identify three classes of compounds with potential ACE-inhibitory activity: flavonoids, procyanidins, and peptides [24].

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The in vitro ACE-inhibiting activity of quercetin demonstrated in the present study was in accordance with values previously reported for this and other flavonoids [11].

We have recently demonstrated that quercetin potentiates the plasma extravasation induced by substance P in the rat urinary bladder through the inhibition of NEP [25]. In the present study, treatment with quercetin through oral and intravenous routes increased the hypertensive response to intravenous administration of BK and reduced the hypertensive response to AI. The results suggest that the vascular effects of quercetin in anesthetized rats are probably due to changes in the levels of these vasoactive substances secondary to the inhibition of ACE. Further experiments are necessary in order to clarify the molecular mechanisms by which quercetin exerts these effects.

References