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Research Paper

Inhibitory effect of quercetin on rat trachea contractility *in vitro*

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Abstract

Objectives The effect of quercetin, a naturally occurring flavonoid traditionally used to treat airway diseases such as bronchial asthma, on the contractile response elicited by electrical field stimulation or carbachol in rat isolated trachea was investigated.

Methods Isolated tracheal tissue was subjected to contractions by an electrical field stimulation of 5 Hz for 30 s, 400 mA, and the responses in the presence of cumulative concentrations of quercetin (10^{-6}–3×10^{-4} M) were observed. The effect of quercetin was also evaluated after administration of phentolamine plus propranolol (to block α- and β-adrenergic receptors), N^G-nitro-L-arginine methyl ester (to block nitric oxide synthesis), capsaicin (to desensitise sensory C fibres), α-chymotrypsin (a proteolytic enzyme that rapidly degrades vasoactive intestinal peptide), SR140333 and SR48968 (tackykinin NK_1 and NK_2 receptor antagonists, respectively).

Key findings Quercetin produced a concentration-dependent inhibition of contractions induced by both carbachol and electrical field stimulation. However, quercetin was more active in inhibiting the contractions produced by electrical field stimulation than those induced by carbachol, suggesting a presynaptic site of action (in addition to a postsynaptic effect, as revealed by the inhibitory action of quercetin on carbachol-induced contractions). The inhibitory effect of quercetin on contractions induced by electrical field stimulation was unaffected by phentolamine plus propranolol, SR 140333 and SR 48968, capsaicin treatment or by the proteolytic enzyme α-chymotrypsin. In contrast, the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester significantly reduced the inhibitory effect of quercetin on contractions induced by electrical field stimulation.

Conclusions Quercetin inhibits rat tracheal contractility through a presynaptic (involving nitric oxide) and a postsynaptic site of action.

Keywords asthma; flavonoids; nitric oxide; quercetin; trachea

Introduction

Flavonoids are plant pigments responsible for the autumnal burst of colour and the many shades of yellow, orange and red in flowers. They also have a role in the normal growth, development and defence of plants. Flavonoids are important constituents of the human diet, being abundant in foods and beverages of plant origin, such as fruits, vegetables, tea, cocoa and wine.[1] Flavonoids are also found in several medicinal plants, and herbal remedies containing flavonoids such as quercetin (e.g. *Ginkgo biloba*, *Grindelia camporum*, *Marrubium vulgare*) have been used in folk medicine for the treatment of several diseases, including those affecting the respiratory tract.[1,2] Among the many different flavonoids present in plants, quercetin is the most abundant.[3,4] Quercetin shows a wide range of biological activities, including inhibition of Na^+/K^+ ATPase, histamine-release, protein kinase C, tyrosine kinase, angiogenesis, angiotensin-converting enzyme II and intestinal peristalsis. Moreover, antioxidant, anticarcinogenic, antihypertensive, anti-inflammatory, pro-apoptotic, hepatoprotective effects, a superoxide dismutase-like activity and modulation of cell cycle have also been reported for quercetin.[5–11] Quercetin has also been proposed for the treatment of chronic rhinosinusitis.[12]

Previous studies have shown that quercetin exerts inhibitory effects on rat vas deferens,[13] vascular tissues[14] and intestinal contractility.[15] Notably, a structure–activity relationship for a number of flavonoids, including quercetin, has been also reported.[14,16] In the present

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study, we investigated the effect and the possible mode of action of quercetin on rat tracheal contractility. For this purpose, we evaluated the effect of quercetin on the nerve contractions induced by electrical field stimulation (EFS) and myogenic contractions induced by carbachol.

Material and Methods

Drugs

Quercetin, carbachol hydrochloride, atropine sulfate, hexamethonium chloride, phentolamine hydrochloride, propranolol hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME), α-chymotrypsin and tetrodotoxin were purchased from Sigma (Milan, Italy); capsaicin was obtained from Tocris Cookson (Northpoint, UK); SR 140333 (*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl-]ethyl}-4-phenyl-1-axoniabicyclo[2,2,2,]octane chloride and SR 48968 (*S*)-*N*-methyl-*N*[4-(4-acetylamono-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide hydrochloride were a gift from SANOFI-Recherche (Montpellier, France). Quercetin was dissolved in dimethylsulfoxide (DMSO) and SR140333 in DMSO/water (50%, v/v). The other drugs were dissolved in distilled water. DMSO (<0.01%) did not modify the contractions induced by EFS or carbachol.

Trachea preparation and drug administration

Male Wistar rats (200–220 g) were purchased from Harlan Italy (S Pietro al Natisone, UD, Italy) and maintained under controlled conditions of temperature (24 ± 2°C) and humidity (60%) until used. Rats had free access to water and food. All experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Rats were killed by asphyxiation with CO_2. The trachea was rapidly removed and transverse rings (3 mm long), including the epithelium, were cut and then mounted in thermostatically controlled (37°C) organ baths. The organ baths contained 20 ml Krebs solution (composition in mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose 11) and were equilibrated with 95% O_2, 5% CO_2. The tissues were connected to an isometric transducer (load 0.5 g) connected to a PowerLab system (Ugo Basile, Comerio, Italy). All experiments commenced after a minimum 60-min equilibration period and the tissues were subjected to contractions induced by an EFS of 5 Hz for 30 s, 400 mA, delivered via electrodes placed around the tissue. Stable and reproducible contractions for a time period of 4 h were obtained, with stimulation every 2 min. After stable control contractions evoked by EFS, the responses were observed in the presence of increasing cumulative concentrations of quercetin (10^{-6}–3×10^{-4} M).

The contact time for each concentration of quercetin was 10 min. Preliminary experiments showed that quercetin reached its maximal inhibitory effect within this time period.

The effect of quercetin (contact time 30 min) was also evaluated after administration to the bath of: phentolamine (10^{-6} M) plus propranolol (10^{-6} M) to block α- and β-adrenergic receptors; L-NAME (3×10^{-4} M) to block nitric oxide (NO) synthesis; capsaicin (3×10^{-5} M) to deplete sensory C-fibre endings; α-chymotrypsin (1 U/ml), a proteolytic enzyme that rapidly degrades vasoactive intestinal peptide; SR140333 (10^{-7} M) to block tackykinin NK_1 receptors; and SR48968 (10^{-6} M) to block tackykinin NK_2 receptors. The concentrations of antagonists/inhibitors used here were selected on the basis of previous studies.[17–22] In preliminary experiments, the effect of tetrodotoxin (3×10^{-7} M), atropine (10^{-6} M) or hexamethonium (10^{-6} M) on EFS-induced contractions was evaluated after a contact time of 15 min.

The effect of quercetin (contact time 10 min) was also evaluated on the contractions produced by carbachol (10^{-7} M). This concentration of carbachol gave a contractile response that was similar in amplitude (i.e. ~0.4–0.5 g) to that obtained by EFS. Carbachol was left in contact with the tissue for 30 s and then washed out. Carbachol-induced contractions were abolished by atropine (10^{-6} M), but unaffected by tetrodotoxin (3×10^{-7} M).

Statistical analysis

Results are expressed as means ± SEM and were analysed by analysis of variance. The concentration of quercetin that produced 50% of the maximal inhibition of EFS-induced contractions (IC50) (geometric mean ± 95% confidence limits) were calculated by nonlinear regression analysis using the equation for a sigmoid concentration–response curve (GraphPad Instat program, version 4.01). A value of $P < 0.05$ was considered significant.

Results

Contractile responses evoked by EFS (5 Hz for 30 s, 400 mA) on the rat trachea were completely abolished by the neural sodium channel blocker tetrodotoxin (3×10^{-7} M) and by the muscarinic receptor antagonist atropine (10^{-6} M). These results confirm the neuronal origin and the cholinergic nature of the response. In addition, these contractions were not significantly modified by 10^{-6} M hexamethonium (nicotinic receptor antagonist), 10^{-7} M SR144333 or 10^{-6} M SR48968 (antagonists of NK_1 and NK_2 receptors, respectively), 1 U/ml α-chymotrypsin (proteolytic enzyme that degrades vasoactive intestinal peptide), 3×10^{-5} M capsaicin (which depletes sensory C-fibre endings), 10^{-6} M phentolamine plus 10^{-6} M propranolol (α- and β-adrenergic receptor antagonists, respectively) or 3×10^{-4} M L-NAME (NO synthase inhibitor) (% variation: SR144333 −20 ± 6.5; SR48968 −13 ± 4; α-chymotrypsin 20 ± 7; capsaicin 7 ± 4; phentolamine plus propranolol 3 ± 4; L-NAME 5 ± 3; n = 6–7 experiments for each antagonist/inhibitor).

Quercetin (10^{-6}–3×10^{-4} M) produced a concentration-dependent inhibition of contractions induced by both EFS and carbachol (Figure 1). Quercetin was significantly more active in inhibiting the EFS-induced contractions than the carbachol-induced contractions (see Figure 1). The IC50 values (95% confidence limits) were 1.45×10^{-5} M (6.28×10^{-6} to 3.35×10^{-5}) for the inhibition on EFS-induced contractions and 4.02×10^{-5} M (1.76×10^{-5}–9.17×10^{-5}) for the inhibition on carbachol-induced contractions.

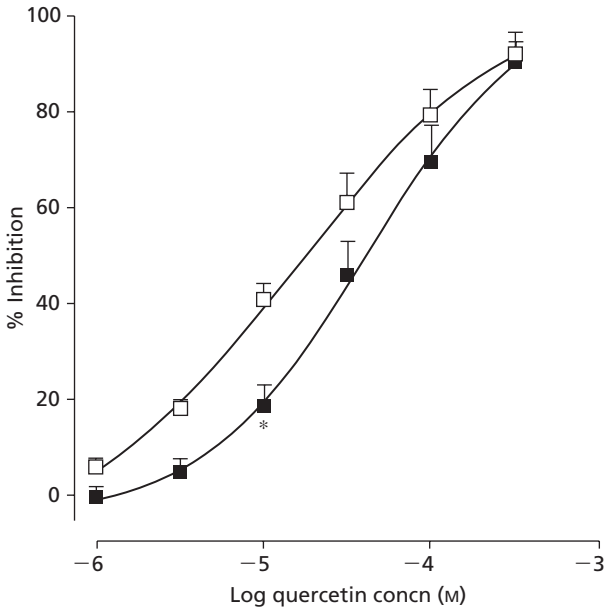

Figure 1 Effect of quercetin on the tracheal contractility induced by electrical field stimulation or carbachol. Inhibitory effect of quercetin (10^{-6}–3×10^{-4} M) on the contractile response produced by electrical field stimulation (□) or carbachol (10^{-7} M; ■) in rat isolated trachea. Each point represents the mean of seven or eight animals. Error bars indicate the SEM. $*P < 0.05$, the inhibitory effect of quercetin on carbachol-induced contractions was significantly different compared with the inhibitory effect of quercetin on contractions induced by electrical field stimulation.

The inhibitory effect of quercetin in the EFS-induced contractions was unaffected by the tachykinin NK_1 antagonist SR144333 (10^{-7} M), the tackykinin NK_2 antagonist SR48968 (10^{-6} M), α-chymotrypsin (1 U/ml), capsaicin treatment (3×10^{-5} M, to desensitise sensory C fibres) and by a combination of drugs that block α- and β-adrenergic receptors (i.e. phentolamine 10^{-6} M plus propranolol 10^{-6} M) (Table 1). However, treatment of the tissue with the NO synthase inhibitor L-NAME (3×10^{-4} M) reduced the inhibitory response of quercetin on EFS-induced contractions (Figure 2).

The vehicle (DMSO 2 μl/20 ml) did not modify EFS-induced contractions ($2 \pm 3\%$ inhibition, $n = 6$, $P > 0.2$).

Discussion

Quercetin reduced both EFS- and carbachol-induced contractions in rat isolated trachea in a concentration-dependent manner. Because the final effector of both carbachol and EFS-induced contractions is the muscarinic receptor located on smooth muscle cells, these results suggest that one of the sites of action of quercetin lies in smooth muscle cells (i.e. postsynaptic site of action). Furthermore, quercetin was more effective at reducing EFS-induced contractions (which are mediated by the release of acetylcholine from postganglionic cholinergic nerves) than carbachol-induced contractions (which are due to a direct activation of muscarinic receptors located on smooth muscle cells). The different potency of quercetin in inhibiting contractions (EFS-induced contractions compared with carbachol-induced contractions) could indicate that quercetin is able to activate receptors or release endogenous substances that have an inhibitory effect on EFS-induced contractions (i.e. quercetin inhibits EFS-induced contractions via a presynaptic site of action). Others have previously demonstrated the antispasmodic action of the quercetin analogue 3-*O*-methylquercetin in guinea-pig isolated trachea.[23]

NO is an important messenger molecule involved in many physiological and pathological processes.[24] NO performs a major role in inhibitory non-adrenergic non-cholinergic transmission,[25–27] as it relaxes vascular and non-vascular smooth muscles of various organs systems such as the airway and the gastrointestinal tract.[28] A major source of NO in the airway is the respiratory epithelium,[29,30] which is relevant given that our experiments were performed with tracheal rings containing the epithelium. Sekizawa *et al.*[17] showed that NO released during EFS may mediate prejunctional inhibition of cholinergic contraction in the rat trachea. We have shown that L-NAME, an inhibitor of NO synthase, reduces the inhibitory effect of quercetin in rat trachea, suggesting a modulatory role of NO in the quercetin-induced reduction of tracheal contractility. Our results are in accordance with previous studies showing the involvement of NO in quercetin-induced effects in other tissues, such as the aorta and the intestine.[10,14,31,32] Moreover, quercetin has been shown to inhibit NO synthase in bovine aortic endothelial cells[33] and to modulate endothelium-derived NO bioavailability in diabetic rat aortas.[34]

Table 1 Effect of quercetin on tracheal contractility induced by electrical field stimulation

Drugs	Concentration (M)					
	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}	10^{-4}	3×10^{-4}
Vehicle (10^{-6}–3×10^{-4} M)	5.5 ± 1.7	17.8 ± 1.8	40.6 ± 3.6	61.0 ± 6.0	79.3 ± 5.3	91.9 ± 4.5
SR144333 (10^{-7} M)	6.3 ± 3.4	11.9 ± 3.8	27.2 ± 7.2	48.2 ± 5.7	76.9 ± 3.8	95.5 ± 1.5
SR48968 (10^{-6} M)	0.5 ± 2.9	7.1 ± 4.4	26.6 ± 7.5	59.9 ± 6.2	81.8 ± 3.4	95.7 ± 1.4
α-Chymotrypsin (1 U/ml)	5.2 ± 5.2	17.2 ± 6.3	36.1 ± 10	67.6 ± 1.2	84.4 ± 1.5	94.5 ± 0.7
Capsaicin (3×10^{-5} M)	8.0 ± 3.6	14.5 ± 4.6	35.7 ± 5.1	59.6 ± 8.9	80 ± 6.0	95.2 ± 1.6
Phentolamine (10^{-6} M) + propranolol (10^{-6} M)	8.6 ± 1.9	18.2 ± 3.5	36.1 ± 5.4	63.8 ± 7.5	82.7 ± 4.2	96.6 ± 2.7

Inhibitory effect of quercetin (vehicle) alone or in the presence of SR144333, SR48968, α-chymotrypsin, capsaicin, or of a combination of phentolamine and propranolol. Results (means ± SEM of seven or eight animals for each antagonist/inhibitor) are expressed as % inhibition of the corresponding control. No significant differences were observed.

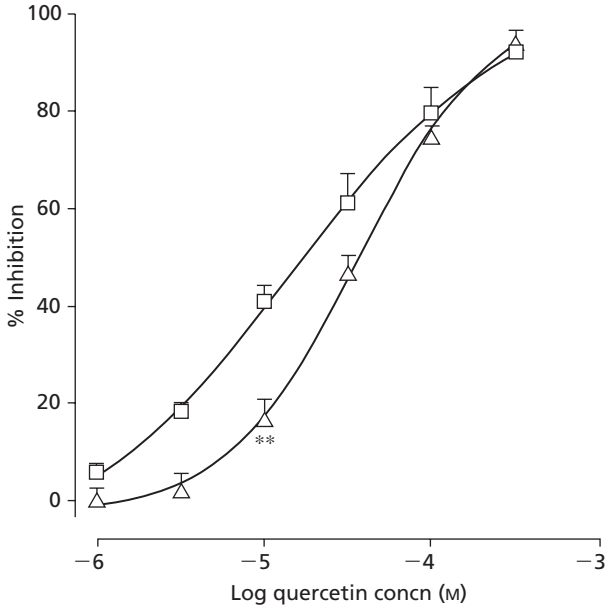

Figure 2 Effect of quercetin alone or in the presence of N^G-nitro-L-arginine methyl ester on tracheal contractility induced by electrical field stimulation. Inhibitory effect of quercetin (10^{-6}–3×10^{-4} M) (vehicle; □) or quercetin in the presence of 3×10^{-4} M N^G-nitro-L-arginine methyl ester (L-NAME; △) (to block NO synthesis). Each point represents the mean of seven or eight animals. Error bars indicate the SEM. $^{**}P < 0.01$, significantly different compared with the vehicle (significant difference between the L-NAME and the vehicle curves).

In a different series of experiments, we excluded the main systems involved in tracheal contractility as potential contributory mechanisms involved in the quercetin action on rat trachea. Specifically, we excluded the involvement of: (1) the adrenergic system, as phentolamine and propranolol did not modify the inhibitory effect of quercetin; (2) tachykinins, as tachykinin NK_1 and NK_2 receptor antagonists did not modify the effect of quercetin; (3) neuropeptides such as vasoactive intestinal peptide, since α-chymotrypsin, a peptidase inhibitor, did not affect the concentration–response curve of quercetin; and (4) sensory nerves, as desensitisation with capsaicin did not modify quercetin-induced inhibition of EFS-induced contractions.

Conclusions

Quercetin inhibits rat tracheal contractility through post-synaptic and presynaptic mechanisms. The presynaptic mechanism involves, at least in part, NO production. Quercetin could be further investigated for possible application in airways diseases such as asthma.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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