Quercetin Inhibits Anaphylactic Contraction of Guinea Pig Ileum Smooth Muscle

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Abstract

Certain flavonoids inhibit antigen-induced release of histamine from mast cells and basophils and also inhibit contraction of guinea pig ileum induced by histamine, acetylcholine, and PGE₂. We examined the effect of one flavonoid, quercetin, on anaphylactic smooth muscle contraction of ileum from guinea pigs sensitized to egg albumin. Quercetin inhibited both the phasic and tonic components of anaphylactic contraction in a concentration-dependent fashion (IC₅₀ approximately 10 μ M). Whether this is primarily an effect on mast cell mediator release or inhibition of mediator effects on smooth muscle has not been established.

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Quercetin, a pentahydroxyflavone, belongs to a class of ubiquitous plant compounds known as flavonoids [1] and is structurally related to the antiallergic drug cromolyn sodium. These compounds are of interest because of the antiallergic properties which have been ascribed to them. For example, the flavonoid d-catechin prevents anaphylaxis in sensitized guinea pigs challenged with horse serum [2]. Derivatives of phloretin inhibit anaphylactic histamine release from passively sensitized human lung tissue [3]. Quercetin inhibits ragweed antigen-induced histamine release from human basophils of subjects with hay fever [4, 5] and also from rat mast cells [6, 7]. Quercetin and certain other flavonoids inhibit guinea pig ileal smooth muscle contraction induced by the agonists histamine, acetylcholine, and PGE₂ [8]. We examined the effect of quercetin, the most active flavonoid in the basophil system, on anaphylactic contraction of guinea pig ileal longitudinal smooth muscle.

Hartley guinea pigs were anaphylactically sensitized by subcutaneous administration in the lower back of 1 ml of a suspension of 100 μ g of egg albumin (5 times recrystallized, Calbiochem, La Jolla, Calif.) in complete Freund's adjuvant (Difco Labs, Detroit,

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Mich.) [9]. Anaphylactic sensitivity was present 3 weeks after injection.

Hartley male albino guinea pigs weighing 300–500 g were stunned and exsanguinated and the terminal 25 cm of ileum was removed and placed in Tyrode's solution at 37 °C. The longitudinal muscle was removed from the underlying circular muscle by a method similar to that described by Rang [10]. The muscle was cut into 2.5-cm strips that were individually suspended in 10-ml jacketed glass tissue baths containing Tyrode's buffer maintained at 37 °C and gassed with 95% O₂:5% CO₂. The Tyrode's solution was of the following composition (millimolar): NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 2.1, NaH₂PO₄ 0.42, NaHCO₃ 12.0, and glucose 5.0 and was adjusted with 107V sodium hydroxide to give a final bath pH of 7.2–7.35. The lower end of the

muscle was fixed and the upper end was threaded to the lever of a force displacement transducer (Grass Instruments FT03C, Grass Medical Instruments, Quincy, Mass.) with a resting tension of 300 mg. Contractile responses were recorded isometrically with an ink writing polygraph (Grass Instruments, Model 5D). Tracings were recorded on curvilinear paper at 0.5 mm/s. Tissues were equilibrated in Tyrode's solution for 60 min with changes every 15–20 min. Phasic contraction was defined as the point of initial maximal pen deflection

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$E^{3/4}_{4/3/4}$ Phasic

Tonic

50

5 10 20

Quercetin concentration, μM

Fig. 1. Graphic representation of data shown in table I.

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Fig. 2. Log concentration-response relationship of data in table I (for the phasic component of anaphylactic contraction).

Table I. Effect of quercetin on anaphylactic smooth muscle contraction

a Mean $\pm$ SEM. Six experiments.

b $p < 0.001$ compared to control contraction (100%).

and tonic contraction as the postphasic, sustained contraction (higher than resting tension) which was measured arbitrarily at 90 s.

After equilibration in the tissue bath histamine-induced contractions were obtained with each muscle to insure that it was normally responsive. Occasional unresponsive tissues were discarded. Quercetin was dissolved in DMSO (Sigma Chemical Co., St. Louis, Mo.) and the maximum final bath concentration did not exceed 0.25%. DMSO at this concentration had neither a direct effect on the muscle nor on anaphylactic contractions. Quercetin was added to one bath to give final concentrations of 5, 10, 20, or 50 μM , and was left in contact with the muscle for 10 min before addition of antigen. Controls consisted of muscle strips of equal length taken from the same segment of ileum. 100 μg of egg albumin was added to the baths yielding a single maximal contraction. Both phasic and tonic responses were measured as millimeters of pen deflection and the effect of quercetin on anaphylactic contraction was expressed as percent of control contraction height obtained in the absence of quercetin. Statistical analysis consisted of the two-tailed t test and a p value of less than 0.05 was accepted as indicative of a significant difference between two sets of observations.

Quercetin inhibited anaphylactic contraction of guinea pig ileum in a concentration-dependent fashion (table I). Phasic contractions were significantly less than control for all concentrations of

quercetin except 5 μ M. Tonic contractions were significantly less than control at concentrations of 20 and 50 μ M quercetin. Linear regression analysis indicated an IC₅₀ of approximately 10 μ M (fig. 1,2).

Based on quercetin's known effects on basophil [4,

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5] and rat mast cell [6, 7] histamine release and agonist-induced smooth muscle contraction [8], it is perhaps not surprising that it was found to be a potent inhibitor of anaphylactic smooth muscle contraction. It is not clear whether this is due primarily to an effect on mediator release, mediator action, or both. However, two bits of evidence support the contention that inhibition of mediator release is the primary mode of action: (1) d-catechin prevented anaphylaxis in sensitized guinea pigs but did not inhibit histamine shock in the same animals [2], and (2) the concentration-response relationship in the present experiments is very similar to that found in the basophil histamine release experiments [4] but this could be fortuitous.

Mobilization of Ca²⁺ and an increase in cytosolic Ca²⁺ concentration is required to trigger smooth muscle contraction [11] and it has been shown that the phasic and tonic components of contraction rely mainly on membrane-bound and extracellular Ca²⁺, respectively [12].

Therefore, quercetin must act in some fashion to reduce the availability of Ca²⁺ to the contractile machinery of smooth muscle cells but the precise locus (or loci) of action remains to be identified.

Quercetin is known to have inhibitory effects on a number of enzyme systems including phospholipase A₂, [13], catechol-O-methyltransferase, cyclic nucleotide phosphodiesterases, and adenosine triphosphatases, amongst others [cf. 5]. Conceivably quercetin (and other flavonoids) may inhibit some crucial enzymes involved in cell activation due to different stimuli including anaphylactic and agonist-induced smooth muscle contraction.

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