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FLAVONOID INHIBITION OF HUMAN BASOPHIL HISTAMINE RELEASE STIMULATED BY VARIOUS AGENTS*

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Abstract—Eleven naturally occurring flavonoids representing five different chemical classes were studied for their effects on human basophil histamine release triggered by six different stimuli. The flavonoids included flavone, quercetin, taxifolin, chalcone, apigenin, fisetin, rutin, phloretin, tangeretin, hesperetin, and naringin. The stimuli were antigen, anti-IgE, concanavalin A, ionophore A23187, formylmethionylleucylphenylalanine, and tetradecanoyl phorbol acetate. Concentration–effect relationships were established for each flavonoid (5–50 μM) at concentrations of stimuli which produced near optimal histamine release. Variable degrees of inhibition were noted depending on the nature of the stimulus and flavonoid structure. The flavonols, quercetin and fisetin, and the flavone, apigenin, exhibited a predilection to inhibit histamine release stimulated by IgE-dependent ligands (antigen, anti-IgE, and con A). The flavanone derivatives, taxifolin and hesperetin, were inactive, as were the glycosides, rutin and naringin. The open chain congeners, chalcone and phloretin, also possessed inhibitory activity. Thus, the flavonoids may be useful probes in comparative analysis of secretory phenomena. The findings suggest that the biochemical pathways leading to secretion differ subtly from one stimulus to another. The differences are detectable with flavonoids of different structures and possibly reflect distinct pathways of Ca^{2+} mobilization or other unique mechanisms of action.

The flavonoids comprise a large group of naturally occurring low molecular weight substances widely distributed in the vegetable kingdom [1, 2]. They serve a multiplicity of functions in plant physiology [1] and have been shown to possess antiinflammatory, antiallergic, antiviral and even mutagenic and anticarcinogenic activities [2–5]. In addition, they may affect the activities of many enzyme systems involved in mammalian physiology [3, cf. Ref. 5]. All flavonoids are derived from the basic structure flavone (2-phenylchromone, or 2-phenylbenzopyrone) which is structurally related to the antiallergic drug cromolyn.

Quercetin and other flavonoids inhibit histamine release from rat mast cells induced by antigen, concanavalin A (con A‡), and the Ca^{2+} ionophore A23187 [6–8]. We have described in detail the properties of quercetin with respect to its ability to inhibit antigen-induced histamine release from human basophils [9] and also have analyzed the effects of a variety of flavonoids in this system in order to establish the structural features necessary for inhibitory activity [10]; quercetin was always the most active compound.

In the present study we examined the effects of several flavonoids of different chemical classes on histamine release from human basophils induced by different immunologic and nonimmunologic stimuli (antigen, anti-IgE, con A, Ca^{2+} ionophore A23187, the chemoattractant peptide, f-MetLeuPhe, and the tumor promoting phorbol ester, tetradecanoyl phorbol acetate) in order to determine the profile of inhibition of histamine release caused by the different flavonoids. The data indicate that flavonoid inhibition of histamine release varies depending on the stimulus and the particular structure of the flavonoid.

MATERIALS AND METHODS

Chemicals. Hesperetin, phloretin, naringin, and flavone were obtained from the Sigma Chemical Co., St. Louis, MO; quercetin, fisetin, rutin, and chalcone from the Aldrich Chemical Co., Milwaukee, WI; apigenin and taxifolin from Tridom Fluka, Hauppauge, NY; and tangeretin as a gift from Dr. John Attaway, Department of Citrus, State of Florida, Lakeland, FL. All flavonoids were dissolved in dimethyl sulfoxide (DMSO, Sigma) and stored at −20° until use when they were diluted in Tris buffer

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‡ Abbreviations: con A, concanavalin A; DMSO, dimethyl sulfoxide; f-MetLeuPhe (or f-MLP), formylmethionylleucylphenylalanine; and TPA, tetradecanoyl phorbol acetate.

(25 mM) containing NaCl (120 mM), KCl (5 mM), Ca^{2+} (0.6 mM), Mg^{2+} (1.0 mM) and 0.03% human serum albumin (Tris-ACM) [11].

Preparation of leukocytes. All studies were performed with leukocyte suspensions obtained from subjects with ragweed hay fever (compatible history and positive prick test with 1:20 ragweed extract). The leukocyte suspensions were prepared as previously described [9, 10] according to May *et al.* [12]. All incubations were carried out at 37° and all experiments were conducted in polypropylene tubes. The concentration–effect curve for each stimulus was determined for all leukocyte donors in preliminary experiments. The concentration of stimulating agent which produced maximal or near maximal histamine release for each donor was used in all subsequent experiments examining the effects of flavonoids; at least three experiments with each flavonoid were conducted using cells of different donors. As a matter of routine [9, 10], the flavonoids were added to the leukocyte suspensions (37°) 10 min before addition of the stimulating agent. Incubations were then continued for 40 min at 37°. Following centrifugation (4°, 10 min, 150 *g*) the supernatant fractions were collected for determination of histamine.

Measurement of histamine. Histamine was determined by the automated spectrophotofluorometric method of Siraganian [13]. Total histamine was measured in untreated leukocyte suspensions, and the histamine content of leukocyte supernatant fractions was determined and results expressed as percent inhibition of histamine release. Control spontaneous histamine release never exceeded 5%. None of the flavonoids nor any of the stimulating agents (or DMSO) interfered with the analytical technique for histamine at the concentrations employed.

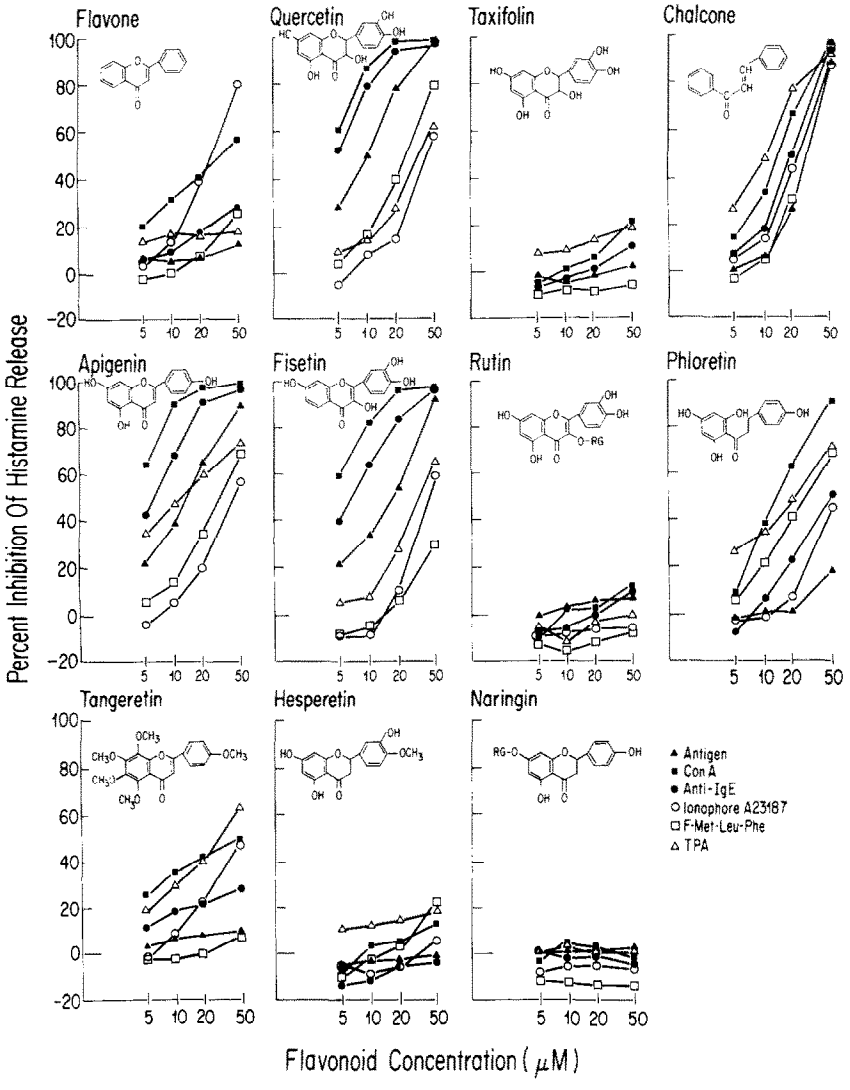

Fig. 1. Structures and inhibitory activities of eleven flavonoids against basophil histamine release stimulated by six different secretogogues. Each point represents the averaged results of at least three experiments (see Table 1). (In the graph for taxifolin the curve for ionophore A23187 virtually overlapped with the data points for antigen and was therefore omitted for clarity). RG = rhamnosylglucoside.

RESULTS

The effects of the flavonoids (5, 10, 20, and 50 μM) on basophil histamine release stimulated by various immunologic and nonimmunologic stimuli are shown in Fig. 1 which graphically displays the pattern of concentration-dependent effects of each of the flavonoids against the individual agents. It is evident that inhibitory activity is dependent upon flavonoid structure and the nature of the particular stimulus, some flavonoids being inactive with some stimuli but active with others (see Discussion).

Table 1 shows the IC_{50} or IC_{25} values for inhibition of histamine release caused by each of the flavonoids. These values were calculated by regression analysis using data points on the inhibition curve which fell between 5 and 95% inhibition. The IC_{25} value was calculated for compounds with weak inhibitory activity against certain secretogogues. By our definition, a flavonoid was considered to be inactive if the measured or calculated IC_{25} value exceeded 100 μM. Depending on structure, a given flavonoid is seen to exert variable degrees of inhibition depending on the nature of the stimulus to histamine secretion.

DISCUSSION

It is evident from the results of these and our earlier experiments [9, 10] that specific flavonoid structural features dictate whether or not a particular flavonoid will exert inhibitory activity against specific stimulus-induced basophil histamine release. This finding is consistent with data reported by Bennett *et al.* [8] on flavonoid inhibition of rat mast cell histamine release and rabbit polymorphonuclear leukocyte release of beta-glucuronidase.

Amongst the flavonoids studied there is considerable structural similarity yet markedly different capabilities to inhibit histamine release stimulated by agents of various natures, both immunologic (antigen and anti-IgE) and nonimmunologic (con A, ionophore A23187, f-MetLeuPhe, and TPA). As can be seen from the structures and activities displayed in Fig. 1 and Table 1 it is evident that the flavonols (3-hydroxy compounds: quercetin and fisetin) and the flavone, apigenin, were the most active inhibitors except that fisetin was relatively inactive against f-MetLeuPhe. Of the three flavones (flavone, apigenin and tangeretin), apigenin was consistently active against each stimulus; flavone and tangeretin, on the other hand, exhibited a wide range of activities being quite active against some stimuli but totally inactive against others. The flavanone, hesperetin, and the flavanonol, taxifolin (dihydroquercetin), showed little or no inhibitory activity against any secretogogue. Thus, saturation of the C2-3 bond (with decreased planarity of the heterocyclic ring) is associated with lack of activity regardless of the stimulus to histamine secretion. Similarly, the glycosides, rutin (a 3-0-glycoside) and naringin (a 7-0-glycoside),

Table 1. IC_{50} or IC_{25} values for flavonoid inhibition of human basophil histamine release stimulated by various agents*

Flavonoid	Inhibitor concentration values (μM)					
	Stimulus					
Structural class	Antigen	Anti-IgE	Con A	A23187	f-MLP	TPA
Flavone						
Flavone	I† (4)	39‡ (4)	34 (4)	24 (5)	48 (3)	I (5)
Apigenin	13 (4)	6 (4)	3 (4)	42 (5)	29 (3)	11 (5)
Tangeretin	I (4)	31 (4)	47 (4)	55 (5)	I (3)	28 (5)
Flavonol						
Quercetin	9 (40)	5 (40)	4 (40)	44 (9)	24 (6)	37 (10)
Fisetin	14 (4)	6 (4)	4 (4)	41 (4)	40 (3)	35 (5)
Flavanone						
Hesperetin	I (4)	I (4)	I (4)	I (4)	62 (3)	I (5)
Flavanonol						
Taxifolin	I (4)	I (4)	69 (4)	I (5)	I (3)	I (5)
Glycosides						
Rutin	I (4)	I (4)	I (4)	I (4)	I (3)	I (5)
Naringin	I (4)	I (4)	I (4)	I (4)	I (3)	I (5)
Chalcone						
Chalcone	25 (4)	18 (4)	13 (4)	20 (5)	24 (3)	10 (5)
Dihydrochalcone						
Phloretin	I (4)	48 (4)	15 (4)	54 (4)	25 (3)	19 (5)

* For all experiments, control histamine release was in the following ranges for each stimulus: antigen, 44.8 to 93.8%; anti-IgE, 16.6 to 54.9%; con A, 15.9 to 52.8%; ionophore A23187, 62.7 to 82.3%; f-MetLeuPhe, 22.8 to 50.2%; and TPA, 18.6 to 60.0%. The concentration ranges of the various ligands utilized to stimulate histamine release were as follows: antigen, 3.65×10^{-3} to 3.65×10^{-2} μg/ml; anti-IgE, 5 to 20 μg/ml; con A, 0.8 to 3.1 μg/ml; ionophore A23187, 0.5 μg/ml; f-MetLeuPhe, 0.5 μM; and TPA, 100 ng/ml.

† I = inactive by our definition, i.e. the measured or calculated IC_{25} value exceeded 100 μM. Numbers in parentheses are the number of experiments performed for each condition.

‡ Underlined numbers indicate IC_{25} values rather than IC_{50} values (see text).

lacked inhibitory activity possibly because of an inability to cross the cell membrane and enter the cytoplasm [14] or because the glycosidic groups sterically hinder a portion of the molecule necessary for inhibitory activity. The open chain flavonoid analogs, chalcone and phloretin, were both quite good inhibitors except that phloretin was inactive against antigen. Between 5 and 50 μM a considerable spread of inhibitory activity can be seen with quercetin, apigenin, fisetin, and phloretin and to a lesser extent with tangeretin, flavone, and chalcone. That is, a strikingly variable sensitivity of different stimuli to inhibition by flavonoids of different structures exists.

With quercetin, fisetin, and apigenin, there is an obvious predilection (Fig. 1) for inhibition of the "IgE-dependent" ligands, i.e. antigen, anti-IgE, and con A (at all concentrations for quercetin). However, it should be noted that the IgE-dependent ligands, antigen and con A, have been shown to differ in the mechanism by which they stimulate basophil histamine release and in their sensitivity to inhibitory agents [15]. It is also of interest that all of the compounds active against receptor-mediated histamine release (especially flavone) were also effective against the non-receptor-dependent secretagogue, ionophore A23187, but whether this "anti-ionophoric" activity represents inhibition of transmembrane Ca^{2+} uptake or inhibition of intracellular Ca^{2+} translocations or some other effect is unknown at present. Indeed, the most striking action of the parent compound, flavone, was inhibition of ionophore-induced histamine release. Of all the flavonoids studied, only the open chain congener, chalcone, caused 85–100% inhibition of histamine release at the highest concentration examined (50 μM) regardless of the nature of the stimulating agent. Based on the results of the present experiments it would appear, therefore, that various secretogogues utilize or activate biochemical pathways leading to secretion that differ subtly from one stimulus to another since certain flavonoids active against one or more stimuli may not be effective against others.

It is generally accepted that calcium ions are the required link in stimulus–secretion coupling in mast cells [16–18] and basophils [11]. Except for TPA, which must mobilize intracellular Ca^{2+} to trigger histamine release [19], all of the stimulating agents studied in the present experiments require extracellular Ca^{2-} to promote the secretory process [20–22] although intracellular sources may also be utilized, as in rat mast cells [23]. However, as discussed by Foreman [24], it is not entirely clear whether different cell membrane stimuli activate ("open") the same or different Ca^{2+} channels or, alternatively, change their permeability characteristics. Nevertheless, the Ca^{2+} sensitivities of anti-IgE- and polyarginine-induced basophil histamine release were significantly different [cf. Ref. 24]. Thus, the data of the present experiments may also be explained by activation of different Ca^{2+} translocation mechanisms by different stimuli. Altered Ca^{2+} translocations could involve transmembrane Ca^{2+} channels and also intracellular pathways of calcium ion movement. That several flavonoids inhibit TPA-induced basophil histamine release (Fig. 1) suggests that these compounds penetrate to the cell interior to cause inhibition. Whether all inhibitory flavonoids act intracellularly and/or inhibit Ca^{2+} uptake, or indeed act by the same or a different mechanism(s) of action is not known.

Although the sequence of biochemical events from stimulus–receptor interaction at the cell surface to final exocytosis of histamine-containing granules is not understood in detail, it is recognized that fusion of granules with each other and with the plasma membrane leads to exocytosis of granule contents to the extracellular milieu [25–30] and that this process presumably involves molecules that act as "fusogens". A number of molecules possess fusogenic properties including fatty acids, lysophosphatides, diacylglycerol and monoacylglycerol [27]. Whether the flavonoids which inhibit histamine release from basophils and mast cells act by inhibiting the formation or the effects of these substances remains to be determined. But it is of interest in this connection that quercetin appears to inhibit the activation of phospholipase A_2 in activated human polymorphonuclear leukocytes as determined by reduction of [^{3}H]arachidonic acid release from prelabeled cells [31, 32] and thus could limit the formation of certain fusogenic molecules derived from phospholipid and arachidonic acid. Along these lines, it is noteworthy that several investigations point strongly to the participation of a lipoxygenase product of arachidonic acid metabolism in the process of histamine release from rat mast cells [33, 34] and basophils [35–37] and that arachidonate metabolites may promote Ca^{2+} uptake [38].

It is important to note, therefore, that antigen-induced histamine release from rat mast cells and guinea pig lung fragments and slow-reacting substance formation from rat mast cells was inhibited by quercetin and also by known inhibitors of arachidonic acid metabolism [5, 8, 11, 14-eicosatetraynoic acid (ETYA) and nordihydroguaretic acid (NDGA)] [39]. These investigators also found that quercetin inhibited the 5-lipoxygenase of rat basophil leukemia cells and human platelet 12-lipoxygenase as well. The data suggest that inhibition of the 5-lipoxygenase may contribute to the inhibition of histamine release and decreased biosynthesis of slow-reacting substance [39]. Data supportive of this contention have also been presented by Showell *et al.* [40].

While we have shown that quercetin inhibition of antigen-induced histamine release from human basophils apparently is not a cyclic AMP-dependent phenomenon [9], it has not been demonstrated in the present experiments that a cyclic AMP-dependent effect is *not* involved in the inhibitory activity of other flavonoids against the secretogogues studied; future experiments will clarify this point. However, the participation of cyclic AMP-dependent protein kinases in rat mast cell histamine release has been strongly suggested in the experiments of Winslow and Austen [41]. The early increase in cyclic AMP in stimulated rat mast cells was associated with activation of two protein kinase isoenzymes. The data suggested that the kinases were involved in the control of both stimulation and inhibition of degranulation. It is perhaps relevant, therefore, that quer-

cetin has been shown to inhibit cyclic AMP-independent protein kinase activity in Ehrlich ascites tumor cells [42] and bovine adrenocortical tissue [43], as well as the tyrosine-phosphorylating protein kinase that is responsible for malignant transformation of Rous sarcoma cells [44]. Whether these findings bear any relationship to protein kinase activation in rat mast cell (or human basophil) histamine release or its inhibition by quercetin is uncertain but quercetin and other flavonoids are reported to affect protein phosphorylation [45, 46].

To date no specific receptor for various flavonoids has been described. Indeed, quercetin, at least, has no effect on resting basophils in that if they are exposed to quercetin (50 μM) for 10–30 min, and then washed, the cells respond to antigen in a perfectly normal fashion with histamine release [9]. Similarly, quercetin inhibits agonist-induced smooth muscle contraction (histamine, acetylcholine, PGE_2) but prior exposure of the muscle to quercetin, followed by washing, does not affect subsequent contractile responses of the muscle to the agonists (unpublished observations); also, tissue culture cell monolayer infectability by certain viruses is not affected by prolonged preincubation with quercetin followed by washing (unpublished observations). These observations are consistent with the report that the effect of quercetin on polymorphonuclear leukocyte oxygen uptake was seen only in con-A-treated [47] or phorbol-myristate-acetate-stimulated [48], but not resting, cells. It would appear, therefore, that only upon activation by some stimulus does the function of a cell become susceptible to modification by a particular flavonoid (at least quercetin and therefore perhaps others). The nature of the process ultimately affected by flavonoids in activated cells has not been determined but, as noted previously, there is evidence that quercetin (and perhaps other flavonoids) may inhibit the activation of phospholipase A_2 and perhaps one or more of the enzymes involved in the lipoxygenase pathways of arachidonic acid metabolism [31, 39, 40, 49].

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