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The activity of compounds extracted from feverfew on histamine release from rat mast cells

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An extract of the plant feverfew (*Tanacetum parthenium*) produces a dose-dependent inhibition of histamine release from rat peritoneal mast cells stimulated with anti-IgE or the calcium ionophore A23187. Greater inhibition of anti-IgE-induced histamine release was achieved with feverfew compared with the inhibition of A23187-induced release. Inhibition of anti-IgE-induced histamine release by feverfew extract was observed when the drug was added simultaneously with anti-IgE and the inhibitory activity increased only slightly when the drug was preincubated with the cells for 5 min before anti-IgE stimulation. In this respect feverfew differs from cromoglycate and quercetin. Feverfew extract inhibited anti-IgE-induced histamine release to the same extent in the absence and presence of extracellular glucose. It is concluded that feverfew extract contains a novel type of mast cell inhibitor.

Feverfew (*Tanacetum parthenium*) has been used as a herbal remedy for inflammatory conditions and migraine since ancient times and there is recent evidence of its effectiveness in migraine (Berry 1984; Johnson et al 1985). In-vitro studies with extracts of feverfew have shown that it inhibits secretion of granules from human platelets (Makheja & Bailey 1981) and from human polymorphonuclear leucocytes (Heptinstall et al 1985). Recent data has indicated that the active principle of feverfew which is responsible for the inhibitory effects on platelets and polymorphonuclear leucocytes, is one or more sesquiterpene lactones containing an α-methylene-butyrolactone unit (Heptinstall et al 1985; Groenewegen et al 1986).

We have recently described the effects of thapsigargin, another sesquiterpene lactone from the plant *Thapsia garganica*, on mast cells, polymorphonuclear leucocytes and platelets (Ali et al 1985). Thapsigargin stimualtes secretion from these cells but we report here that feverfew extracts are inhibitors of histamine release from mast cells.

METHODS

Histamine release

Male Sprague-Dawley rats, 290–370 g, were killed by cervical dislocation following anaesthesia with nitrous oxide. The fur was cut from the abdominal wall and 5 mL of heparinized saline (heparin 25 u mL^{-1}; NaCl 154 mM) was injected into the peritoneal cavity. The peritoneal washings were removed with a Pasteur pipette through a midline incision. The cell suspension was divided into aliquots of 1 mL in polystyrene centrifuge tubes, centrifuged at 120*g* for 5 min and the pellets resuspended in 0·8 mL of Tyrode solution, with or without calcium according to the experimental protocol. The cells were preincubated for 10 min at 37 °C to permit temperature equilibration and then drug added as per protocol. Incubation at 37 °C proceeded for a time specified in individual protocols before the addition of the histamine-releasing agent in a volume of 0·1 mL. A further incubation of 10 min at 37 °C was then allowed for histamine release to occur, the final incubation volume being 1 mL. The reaction was then terminated by quenching each tube with 4 mL ice-cold Tyrode solution. The tubes were centrifuged at 800*g* for 5 min and the supernatants retained for histamine assay. The pellets were resuspended in 5 mL of Tyrode solution and heated on a water bath at 100 °C for 5 min to release any histamine remaining in the cell pellet.

The released and residual histamine was assayed fluorimetrically after condensation with *o*-phthalaldehyde without extraction steps (Shore et al 1959). Histamine released was calculated as a percentage of the total histamine content in each tube. Histamine release occurring in the absence of any releasing agent or drug (spontaneous release; 5·5 ± 0·6% of total) is subtracted from the stimulated release.

The composition of Tyrode solution was: (mM) NaCl 134, KCl 2·7, glucose 5·6, NaH_2PO_4 0·4, HEPES [4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid] 20 mM. Calcium, where required, was added as a small volume of a stock solution of $CaCl_2$ 1 M.

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Preparation of feverfew

The original extract was obtained by extraction of 1 g of air-dried leaves using chloroform (20 mL). This extract was then dried under nitrogen. The extract was reconstituted with 2 mL of chloroform and from this a suitable aliquot was taken. The aliquot was dried under nitrogen and then a volume of Tyrode solution was added, as determined by the experimental protocol. The tube was then vigorously vortexed and incubated for 30 min, with intermittent vortexing, at 37 °C. After 30 min the tube was removed from incubation, vortexed again and then allowed to stand at room temperature (20 °C) for the duration of the experiment.

The extract was prepared by Dr A. Groenewegen, University of Nottingham Medical School. Goat anti-(rat-IgE) serum was obtained from Miles Laboratories Ltd. Quercetin was obtained from May & Baker Ltd and A23187 (2-[(3β,9α,11β-trimethyl)-8-(2-pyrrolecarboxymethyl)-1,7-dioxaspiro[6,6]undecyl-2β-methyl]-5-methylaminobenzoxazole-4-carboxylic acid) from Calbiochem.

RESULTS

The extract of feverfew described in Methods produced a dose-dependent inhibition of histamine release induced by anti-IgE from rat peritoneal mast cells (Fig. 1). The feverfew extract was active over the range of dilutions from 1 in 320 to 1 in 20.

The feverfew extract was almost maximally effective when added together with the anti-IgE stimulus and a slight but statistically insignificant increase in its inhibitory action was observed after a 5 min preincubation of the cells with the extract before addition of the anti-IgE.

Feverfew extract also inhibited histamine release induced by the calcium ionophore A23187 but it was less potent as an inhibitor of histamine release induced by A23187 than as an inhibitor of anti-IgE-induced histamine release (Fig. 2A). A23187 releases a greater fraction of total cell histamine than does a maximally effective concentration of anti-IgE. Since there is evidence that some inhibitors of histamine release are more effective at lower levels of histamine release, one explanation of the relatively weaker effect of feverfew extract against A23187-induced release could be the greater levels of histamine release achieved with A23187. Fig. 2B provides evidence compatible with this explanation. Lowering the extracellular calcium concentration to 0·3 mM from 1 mM reduced A23187-induced histamine release from 60·1% of total to 49·2%, and the percentage inhibition of release by feverfew increased from 4·6 to 30·6% (Fig. 2B). Even so, anti-IgE-induced release in the presence of 1 mM calcium was 33·9% and the same concentration of feverfew inhibited this by 81·1%.

The ability of feverfew to inhibit immunologically stimulated histamine release was compared with the activity of the plant-derived flavonoid, quercetin, which also inhibits histamine release (Fewtrell & Gomperts 1977). Fig. 3 shows the concentration-response relationships for inhibition of histamine release by quercetin and feverfew extract. Quercetin, like cromoglycate, produces maximum inhibition of histamine release when added with the stimulus (anti-IgE) and both drugs are less effective when the cells are preincubated with the drug before addition of the stimulus to histamine release (Kusner et al 1973). Fig. 4 compares feverfew extract, quercetin and cromoglycate with respect to inhibition of histamine release when the drug is added together with anti-IgE or 5 min before anti-IgE stimulation. Both quercetin and cromoglycate are less effective with 5 min preincubation of cells with the drug before anti-IgE stimulation, whereas feverfew extract is active when added with anti-IgE and its activity increases slightly with 5 min of preincubation (Fig. 4).

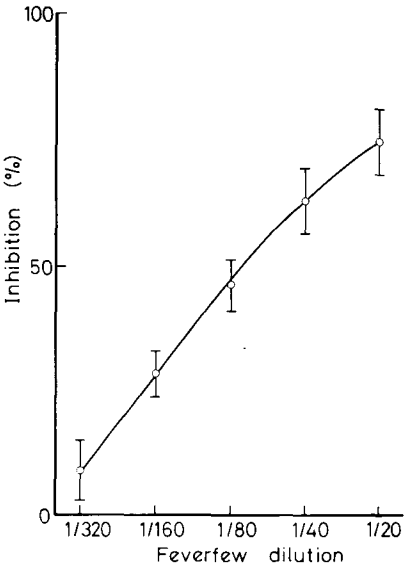

FIG. 1. Concentration-response curve for the inhibition by feverfew extract of histamine release from rat peritoneal mast cells stimulated with anti-IgE (1 : 200 dilution). Each point represents the mean percentage inhibition of a control release of 42 ± 6% of total histamine. Control release was obtained by stimulating the cells with anti-IgE in the absence of feverfew. Vertical bars represent 1 s.e.m. n = 10.

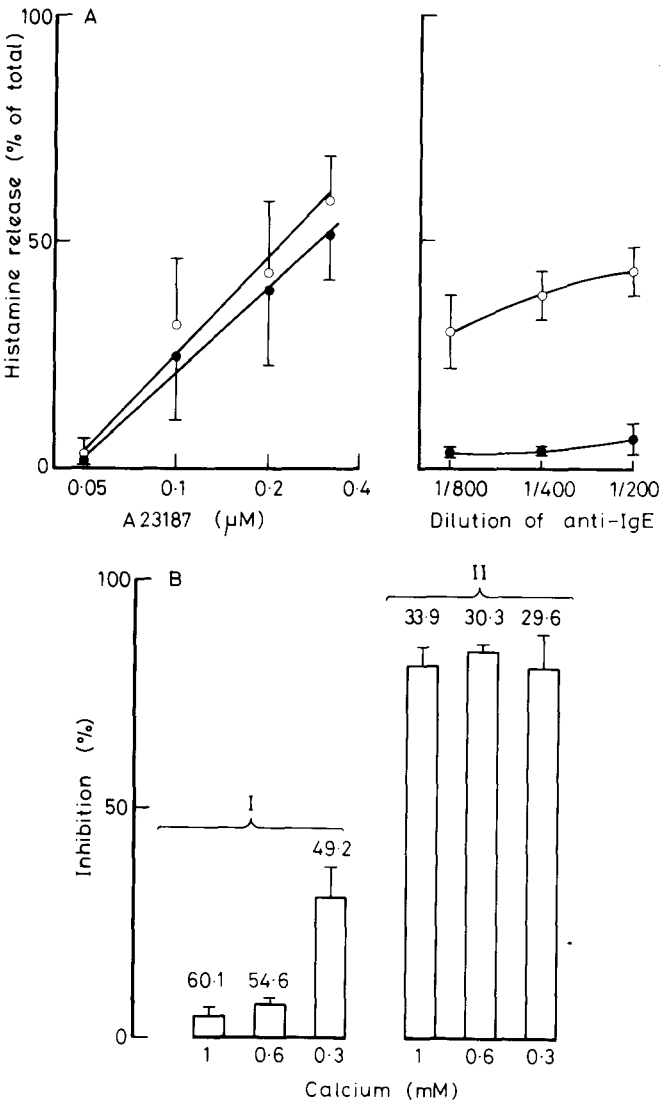

FIG. 2. (A) Effect of feverfew extract (1 : 40 dilution) on the concentration-response relationships for histamine release induced by either A23187 or anti-IgE. Open symbols are the release in the absence of feverfew and closed symbols the release in the presence of feverfew (1 : 40). Each point is the mean of 4 experiments and vertical bars represent 1 s.e.m. Cells were preincubated with feverfew for 5 min before the addition of either A23187 or anti-IgE. (B) Inhibition by feverfew extract (1 : 40 dilution) of histamine release induced by (I) A23187 0·6 μM or (II) anti-IgE, 1 : 200 dilution at various extracellular calcium concentrations. Each column represents the mean ± 1 s.e.m. from 3 experiments. The figure above each column is the histamine release obtained in the absence of feverfew extract. Cells were preincubated with feverfew for 5 min before the addition of either A23187 or anti-IgE.

Histamine release is dependent upon intracellular ATP generation which comes either from glycolysis or oxidative phosphorylation. Inhibitors of oxidative phosphorylation such as antimycin A or cyanide cause almost complete inhibition of immunologically induced histamine release when glucose is absent from the extracellular medium. In the presence of glucose, the inhibition by antimycin A or cyanide is reversed (Johansen & Chakravarty 1972). To determine whether feverfew extract may be acting as a mitochondrial poison we have studied its effect on anti-IgE-stimulated histamine release in the presence and absence of extracellular glucose. Fig. 5 shows that extracellular glucose has no effect on the ability of feverfew to inhibit anti-IgE-induced histamine release.

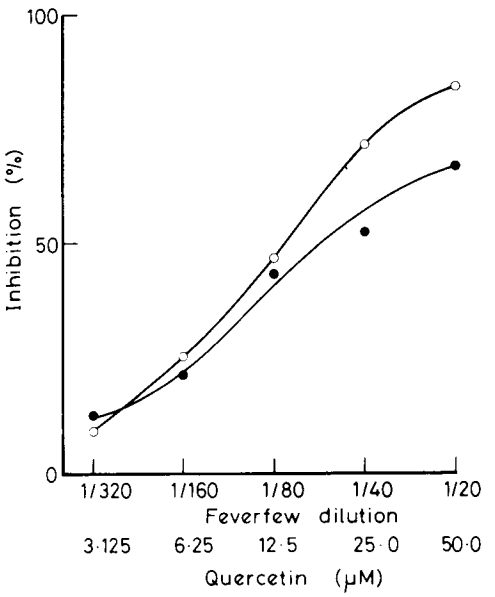

FIG. 3. Concentration-response relationships for the inhibition by feverfew extract (●) or quercetin (○) of histamine release induced by anti-IgE (1:200 dilution). Anti-IgE induced a mean histamine release of 38%. Each point is the mean from 2 experiments. Cells were preincubated with quercetin or feverfew for 5 min before addition of anti-IgE.

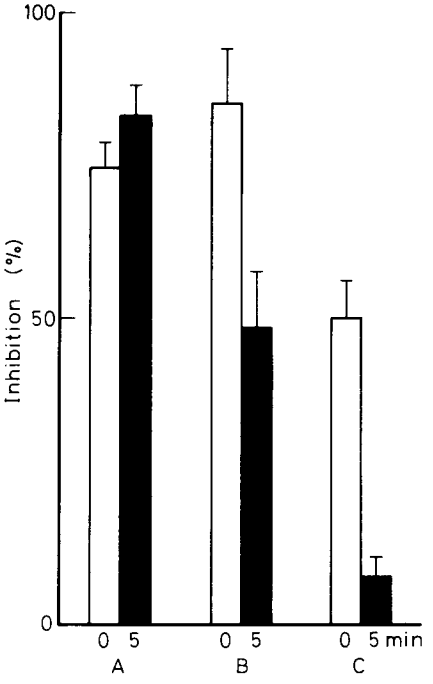

FIG. 4. Effect of preincubation time on the inhibition by (A) feverfew 1/20, (B) quercetin 25 μM, or (C) cromoglycate 20 μM of histamine release induced by anti-IgE (1:200 dilution). Open columns show the inhibition as a percentage of control histamine release achieved by simultaneous addition of anti-IgE and either cromoglycate, quercetin or feverfew. Filled columns show the inhibition achieved by preincubating cells with cromoglycate, quercetin or feverfew for 5 min at 37 °C before addition of anti-IgE. In all cases, release was terminated 10 min after addition of anti-IgE (see Methods). Control release of histamine by anti-IgE in the absence of any inhibitory drug was 55 ± 3% of total cell histamine. The results are from 4 experiments: means and 1 s.e.m. are shown.

DISCUSSION

Feverfew extract contains a compound which produces a concentration-related inhibition of histamine release induced by stimulating rat peritoneal mast cells through an IgE-anti-IgE reaction. The data presented in this paper suggest that this action of feverfew extract is different from the inhibitory action on mast cells of both cromoglycate and quercetin. Furthermore, evidence is presented which suggests that feverfew extract does not inhibit histamine release by interfering with oxidative phosphorylation.

The calcium ionophore A23187 induces histamine release by transporting calcium from the extracellular compartment into the cell (Foreman et al 1973). It has been argued that drugs which inhibit immunologically mediated histamine release, but do not inhibit A23187-induced release, act by preventing a step in the histamine release process at or before the immunologically activated entry of calcium into the cell. Thus, a drug which inhibits the entry of calcium into the mast cell following immunological stimulation has no effect when this route of calcium entry is by-passed by the ionophore. The data in this paper show that feverfew extract does inhibit histamine release induced by the calcium ionophore A23187, although it is less effective against A23187-induced release compared with anti-IgE-induced release. Two explanations for the lesser activity against A23187-induced release may be advanced. Firstly, feverfew extract may exert its principal action at or before the immunologically mediated entry of calcium into the mast cell, with a smaller action on a stage of histamine release which follows calcium entry. Secondly, inhibition of histamine release may be very sensitive to the strength of the stimulus so that the relatively strong stimulus provided by A23187 is less easy to inhibit than the weaker anti-IgE stimulus: there is some evidence for this from other studies on rat mast cells (Pearce & Truneh 1981; Gillespie & Lichtenstein 1975). In platelets and polymorphonuclear leucocytes, feverfew has been shown not to inhibit secretion induced by the ionophore A23187 (Heptinstall et al 1985),

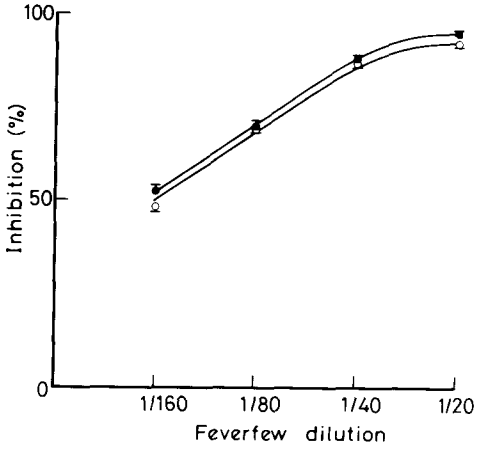

FIG. 5. Effect of removal of extracellular glucose (5·6 mM) on the concentration-response curve for the inhibition by feverfew extract of anti-IgE-induced histamine release. The ordinate is percentage inhibition of anti-IgE-induced release occurring in the absence of feverfew which had a mean value of 54 ± 8% of total cell histamine in the presence of glucose and 48 ± 4·1% in the absence of glucose. ●—● no glucose; ○—○ glucose 5·6 mM. Each point is the mean ± 1 s.e.m. from 3 experiments. The anti-IgE was used at a dilution of 1 : 200.

and this suggests, therefore, that in these systems, feverfew exerts its inhibitory action on the secretory mechanism at some point before the rise in cytosolic free calcium concentration which activates secretion. Our data in rat mast cells are consistent with the observations in platelets and polymorphonuclear leucocytes but our finding that A23187-induced histamine release can be inhibited by feverfew, albeit less effectively than anti-IgE-induced release, does not permit the conclusion that feverfew prevents histamine secretion solely at a point before the rise in cytosolic free calcium concentration.

The fact that feverfew extract contains an inhibitor of mast cell function which differs from cromoglycate, quercetin and mitochondrial inhibitors may have implications for the development of a different class of antiallergic drugs. Further studies will now be aimed at studying the chemical nature of the compounds in feverfew extract which produce this action on rat mast cells.

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