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Antihistaminic activity of pulegone on the guinea-pig ileum

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Abstract—Pulegone, a natural monoterpene compound, has an antihistamine effect on guinea-pig ileum. Its antagonism is of the competitive type ($PA_2 = 6{\cdot}35$) like that of mepyramine and dexchlorpheniramine, two H_1-antagonists, with PA_2 values of 10·15 and 8·74, respectively.

Spasmolytic, antispasmodic and antihistamine properties have been described for a number of monoterpene compounds (Sticher 1977). The terpene pulegone is the major component of the essential oil of *Calamintha sylvatica* subsp. *ascendens*, a plant with antispasmodic and antihistamine properties (Ortiz de Urbina, unpublished results). In the present work, its antihistamine activity on guinea-pig ileum has been compared with that of two H_1-antihistamines, mepyramine (pyrilamine) and dexchlorpheniramine.

Materials and methods

Pharmacological test: guinea-pig ileum. Terminal segments of ileum, about 3 cm in length, were prepared from fasted (48 h) guinea-pigs of either sex (250–400 g) provided by the animalarium of the Faculty of Pharmacy, Salamanca University.

These segments were placed in 25 mL baths with Tyrode solution (NaCl 137, KCl 2·68, $MgSO_4 \cdot 7H_2O$ 1·05, $NaH_2PO_4 \cdot H_2O$ 0·34, glucose 5·55, $CaCl_2$ 1·78, $NaHCO_3$ 11 mM). The solution was kept at 37°C and gassed with 5% CO_2 in O_2. Initial tension was 1 g and the stabilization time was 30–45 min. Isometric contractions were recorded on a Panlab transducer connected to a Panlab Omniscribe recorder.

Increasing concentrations of histamine were added to the bath at 30 min intervals and a control cumulative concentration-response curve was constructed. Pulegone (suspended in Brij 35, 12:1 v/w), mepyramine or dexchlorpheniramine was then added to the bath 5 min before the corresponding concentration-response curve was recorded.

In addition, the antihistamine effects of pulegone, mepyramine and dexclorpheniramine were each evaluated against a fixed, minimally effective dose of histamine ($6{\cdot}7 \times 10^{-8}$ M), in terms of their ability to prevent the histamine contractions when they were added to the bath 5 min before the histamine.

Substances and drugs used. These were: Pulegone (Merck), mepyramine (pyrilamine) maleate (Sigma), dexchlorpheniramine maleate (Schering Corporation), Brij 35 (Merck), histamine dihydrochloride (Prolabo).

Analysis of results. Contractions were expressed as percent of the maximal contraction obtained from the corresponding control curve. On the response curves, each point shows the mean and s.e.m. of six experiments. The cumulative histamine concentration-response curves, with and without the antagonists, was analysed using the Tallarida & Murray (1986) computer program. The EC50, maximum effect (E_{max}), potency ($pD_2 = -\log_{10}$ (EC50)) and affinity (1/EC50) of histamine were determined.

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The antagonist potencies (pA_2) of pulegone, mepyramine and dexchlorpheniramine were also calculated.

The inhibition of the minimal histamine contraction by these antihistamines, i.e. the antagonist potency in terms of a pD_2' value, was also evaluated using the Tallarida & Murray program.

Student's *t*-test was used to estimate statistical significance.

Results and discussion

Pulegone displaced the cumulative histamine concentration-response curves towards higher concentrations, i.e. it increased the EC50 of histamine (Fig. 1), as did mepyramine (Fig. 2) and dexchlorpheniramine (Fig. 3). This displacement increased with

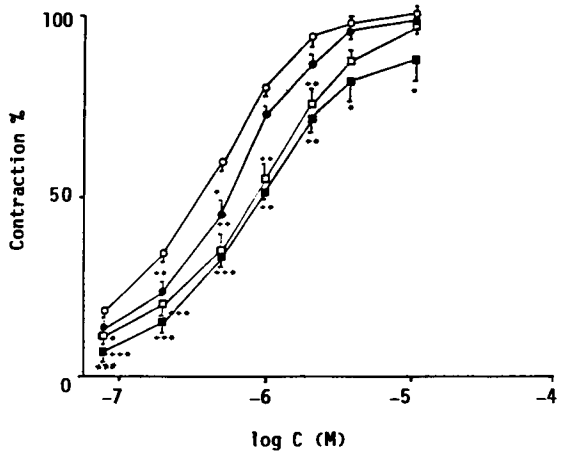

FIG. 1. Effect of increasing concentrations of pulegone on the cumulative concentration-response curves to histamine in the guinea-pig ileum. Control (○), pulegone (●) $2{\cdot}4 \times 10^{-7}$, (□) $4{\cdot}8 \times 10^{-7}$ and (■) $7{\cdot}2 \times 10^{-7}$ M. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

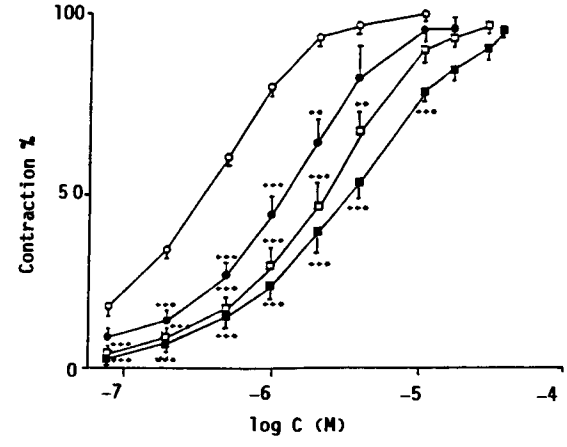

FIG. 2. Effect of increasing concentrations of mepyramine on the cumulative dose-response curves to histamine in the guinea-pig ileum. Control (○), mepyramine (●) $1{\cdot}2 \times 10^{-10}$, (□) $1{\cdot}8 \times 10^{-10}$, (■) $2{\cdot}4 \times 10^{-10}$ M.

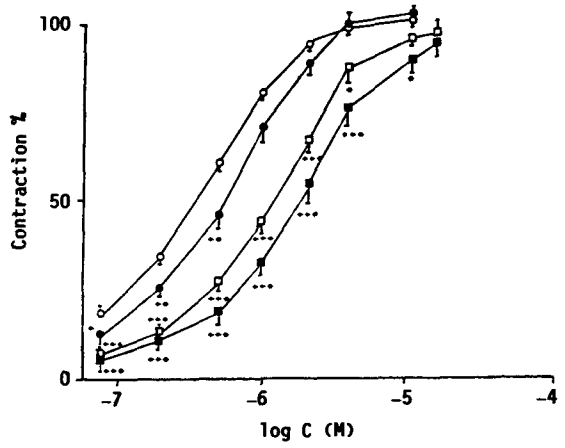

FIG. 3. Effect of increasing concentrations of dexchlorpheniramine on the cumulative dose-response curves to histamine in the guinea-pig ileum. Control (○), dexchlorpheniramine (●) $1{\cdot}3 \times 10^{-9}$, (□) $2{\cdot}6 \times 10^{-9}$, (■) $3{\cdot}8 \times 10^{-9}$ M.

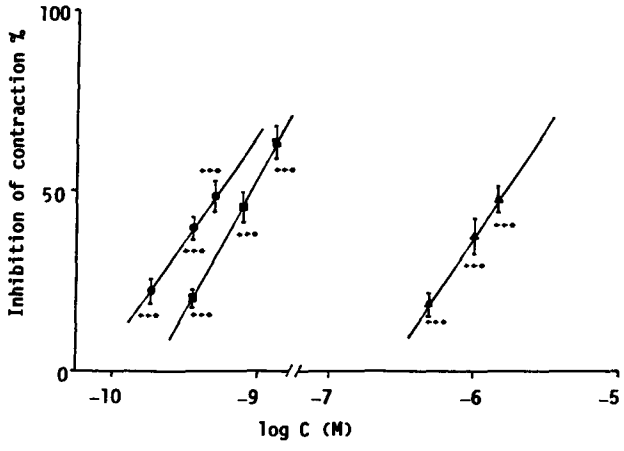

FIG. 4. Regression lines of the inhibitory effect of pulegone (▲), mepyramine (●) and dexchlorpheniramine (■), against histamine ($6{\cdot}7 \times 10^{-8}$ M).

antagonist dose in all three cases, i.e. histamine affinity and potency decreased with increasing doses of pulegone, as they did with the other two drugs. Only one of the nine test curves showed a decrease in E_{max} (that of the highest dose of pulegone). If biologically significant, this small decrease might perhaps reflect a partly non-competitive or irreversible competitive type of antagonism. The essential oil from which pulegone is derived can dramatically reduce E_{max}, and the corresponding response curves are comparable with irreversible competitive antagonism. It is also possible that the essential oil acts non-competitively, since it can inhibit duodenum contractions induced by acetylcholine and barium chloride (our unpublished results).

With the possible exception of the small decrease of E_{max} at maximum dose, pulegone's antagonism of histamine action appears to be competitive, like that of mepyramine and dexchlorpheniramine. Moreover, the regression line slopes from the cumulative histamine concentration-response curves were all close to that for histamine alone ($63{\cdot}89 \pm 1{\cdot}63$), again indicative of competitive antagonism. Slopes for increasing doses of pulegone were $70{\cdot}17 \pm 6{\cdot}81$, $54{\cdot}63 \pm 4{\cdot}32$ and $59{\cdot}67 \pm 3{\cdot}64$, those for mepyramine $59{\cdot}07 \pm 4{\cdot}50$, $61{\cdot}62 \pm 5{\cdot}73$ and $52{\cdot}58 \pm 2{\cdot}61$, and those for dexchlorpheniramine $63{\cdot}95 \pm 5{\cdot}24$, $63{\cdot}37 \pm 3{\cdot}93$ and $70{\cdot}90 \pm 1{\cdot}36$, respectively.

The histamine potency (pD_2) in the control was 6·47, and for increasing doses of pulegone the values were 6·30, 6·11 and 6·06, respectively. In comparison, the pD_2 values for increasing doses of mepyramine were 5·91, 5·64 and 5·49, and those for dexchlorpheniramine were 6·30, 5·93 and 5·75, respectively.

When the antihistamine activity of pulegone was tested against the minimally effective dose of histamine, the results were similar to those of mepyramine and dexclorpheniramine (Fig 4). Also, the pD_2' value for each antagonist (5·81, 9·28 and 9·08, respectively) was similar to the corresponding pA_2 value which had been obtained from the cumulative dose-response curves (6·36, 10·16 and 8·75).

In conclusion, pulegone, a major monoterpene component of the essential oil of *Calamintha sylvatica* subsp. *ascendens* seems to have an antihistaminic activity in the guinea-pig ileum, similar to that of H_1-antihistamines mepyramine and dexchlorpheniramine. However, it is a less potent antagonist than the other two drugs.

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