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Effects of smooth muscle calcium antagonists on human basophil histamine release*

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Calcium antagonists including verapamil, D-600, and nifedipine (BAY 1040) can function as potent inhibitors of vascular smooth muscle contractile responses induced by agonists or by potassium (K^+) depolarization [1–3]. They appear to function as competitive Ca^{2+} antagonists [4–6]. Not all smooth muscle responses are sensitive to these antagonists and it appears likely that the potent inhibitory activity of these compounds (IC_{50} value values of 10^{-6} to 10^{-9} M) is directed to one component of Ca^{2+} translocation, that through voltage-sensitive Ca^{2+} channels [1, 2]. Other smooth muscle relaxants, which include a very diverse collection of structures [7], act at different sites, and in a less defined fashion, to reduce Ca^{2+} translocation [8, 9]. Two of these, dantrolene sodium and 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate (TMB-8), have been found to antagonize contractile responses in guinea pig ileum and vas deferens. Not all smooth muscle contractile responses are blocked to the same degree by different Ca^{2+} antagonists [2].

Calcium is also required for stimulus-secretion coupling in histamine release from rat mast cells and human basophils caused by antigens and mitogens [10–12]. Histamine release from these cells can be inhibited by a variety of agents including beta agonists, E series prostaglandins and choleratoxin, and inhibition is associated with an increase in cellular cyclic AMP content [13]. The Ca^{2+} antagonists TMB-8 [14] and dantrolene sodium [9] inhibit in variable fashion the release of histamine from rat mast cells caused by dextran, compound 48/80, and the calcium ionophore A23187 [15–17]. The flavonoid quercetin is also an active inhibitor of antigen-induced histamine release from human basophils [18]. In the latter system the inhibitory effect of quercetin is partly overcome by increased buffer Ca^{2+} concentrations. In the experiments reported here we compared the "classical" smooth muscle Ca^{2+} antagonists verapamil and nifedipine, as well as dantrolene sodium and TMB-8, with quercetin for their inhibitory activities on antigen-induced histament release from human basophils.

Materials and methods

Chemicals. Verapamil and D-600 were obtained from Knoll, A. G., Ludwigshafen, West Germany, nifedipine from Bayer, A. G., Postsach, Federal Republic of Germany, dantrolene sodium from Norwich-Eaton Pharmaceutical, Norwich, NY, and quercetin from the Aldrich Chemical Co., Milwaukee, WI. TMB-8 was synthesized in our laboratory [19]. All compounds were dissolved in dimethylsulfoxide (DMSO) and were diluted in Tris-buffer

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(25 mM) containing calcium (0.6 mM), magnesium (1.0 mM), and 0.03% human serum albumin (Tris-ACM) [20] prior to addition to leukocyte suspensions.

Preparations of leukocyte suspensions. Leukocyte suspensions largely freed of erythrocytes were prepared from blood of subjects with ragweed hay fever (as determined by history and positive prick skin tests) according to the method of May *et al.* [21]. The concentration of DMSO in the final leukocyte suspensions was 1.0%. This concentration of DMSO did not interfere with antigen-induced histamine release or with the analytical technique for histamine. An aqueous extract of whole ragweed pollen was used to initiate histamine release.

Measurement of histamine. Histamine was determined by the spectrophotoflurometric method [22] as modified [21]. Total histamine was measured in untreated leukocyte suspensions and the histamine content of leukocyte suspensions and of leukocyte supernatant fractions after different experimental manipulations was determined, the results were expressed as the percentage of total histamine released. None of the compounds interfered with the analytical technique for histamine except nifedipine which was, therefore, studied at lower non-interfering concentrations (see below).

Results and discussion

Effects of verapamil, D-600, nifedipine, TMB-8, dantrolene sodium, and quercetin on histamine release. Leukocyte suspensions were preincubated with the potential inhibitors at concentrations of 5–50 μM for 10 min prior to addition of ragweed antigen for a 40-min incubation at 37°. Nifedipine at a concentration of 50 μM interfered with the fluorometric measurement of histamine and so it was studied at 0.01, 0.10, and 1.0 μM concentrations where the interference was not measurable. Following centrifugation in the cold, the supernatant histamine was determined. Table 1 shows the different inhibitory effects of the compounds. In all experiments the Ca^{2+} antagonists were compared with quercetin on the same day. Only quercetin produced a significant concentration-dependent inhibition of antigen-induced histamine release. Nifedipine at the lower concentrations studied failed to exhibit inhibitory activity.

Stimulus–secretion coupling in basophils and mast cells and excitation–contraction coupling in smooth muscles are both Ca^{2+}-dependent processes [11, 23, 24], although the sources for, and mechanisms of, Ca^{2+} translocation are not understood in detail. In fact, it is highly probable that both extracellular and intracellular Ca^{2+} sources may be mobilized according to the cell type and stimulus.

The present experiments indicate that the mechanisms of Ca^{2+} translocation involved in smooth muscle contraction and basophil histamine release are different since the smooth muscle Ca^{2+} antagonists verapamil, D-600, nifedipine, TMB-8, and dantrolene sodium had no inhibitory effect on antigen-induced basophil histamine release. On the other hand, quercetin was a very effective inhibitor but it is an ineffective inhibitor (10^{-6} M) of agonist-induced smooth muscle contraction (D. J. Triggle and E. Middleton, Jr., unpublished observations).

Of particular interest is the inactivity of verapamil, D-600, and nifedipine as inhibitors of basophil histamine release when employed at concentrations significantly higher than those required to block the voltage-sensitive type. A similar conclusion has been reached for a number of other stimulus–secretion coupling systems including mast cells, salivary glands and the exocrine pancreas [2, 11, 25].

Rat mast cells and human basophils may differ in their responses to certain of these agents since TMB-8 and dantrolene were shown to possess some inhibitory activity against histamine release (dextran, 48/80, ionophore) from rat mast cells [15] but not from antigen-stimulated human basophils (Table 1). Quercetin apparently is an equally effective inhibitor of antigen-induced histamine release from human basophils and rat mast cells.

In summary, these observations indicate that the smooth muscle Ca^{2+} antagonists verapamil, D-600, nifedipine, TMB-8, and dantrolene sodium lack activity as inhibitors of antigen-induced histamine release from human basophils, in contrast to the flavonoid quercetin which is an effective inhibitor in basophils but lacks activity in smooth muscle.

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Table 1. Effect of smooth muscle calcium antagonists and quercetin on antigen-induced basophil histamine release

	Percent inhibition of histamine release		
	Concentration (μM)		
Compound	10	20	50
Quercetin	51.1 ± 18.3* (5)	70.7 ± 16.8 (13)	96.7 ± 5.3 (13)
Verapamil	8.0 ± 7.9 (3)	0.1 ± 4.9 (4)	−2.1 ± 11.3 (5)
D-600	−1.5 ± 6.9† (3)	−8.2 ± 6.5 (3)	−4.3 ± 8.1 (5)
Dantrolene	0.3 ± 6.8 (3)	6.1 ± 11.4 (3)	14.5 ± 12.9 (3)
TMB-8	−3.6 ± 6.2 (3)	−8.6 ± 12.4 (9)	−9.3 ± 11.5 (9)
	0.01†	0.1	1.0
Nifedipine	3.1 ± 5.6 (3)	−0.2 ± 7.7 (3)	−0.8 ± 9.8 (3)

* Percent inhibition ±1 S.D. Numbers in parentheses are number of experiments.

† A minus sign indicates enchancement of histamine release.

‡ Lower concentrations of nifedipine were used to reduce interference with the fluorometric measurement of histamine.

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Estrogenic activities of methoxychlor metabolites

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The DDT substitute, methoxychlor [2,2-bis(*p*-methoxyphenyl)-1,1,1-trichloroethane], has been shown to have weak estrogenic activity *in vivo* in experimental animals [1–3]. The parent compound does not have significant affinity for the estrogen receptor in *in vitro* assays [4–7]; however, incubation of methoxychlor with rat liver microsomal preparations in the presence of NADPH increases the ability of methoxychlor to inhibit [^{3}H]estradiol binding to rat uterine receptor [6–9] or to elicit translocation of the receptor to the nucleus [7]. These observations strongly suggest that a metabolite(s) of methoxychlor might account for its *in vivo* estrogenic activity. Two principal metabolites of methoxychlor are the demethylated derivatives [10]: (1) 2-(*p*-hydroxyphenyl)-2-(*p*-methoxyphenyl)-1,1,1-trichloroethane (mono-phenol), and (2) 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (bis-phenol). This communication describes the *in vitro* affinities of these metabolites for the rat uterine estrogen receptor. A preliminary report of this work has appeared [11].

Materials and methods

With the exception of the chemical synthesis of the major methoxychlor metabolites, the materials were as described previously [4]. Following the procedure of Kapoor *et al.* [10], freshly distilled chloral (5 ml) and 7.4 g of aluminum chloride were added to 9.4 g of phenol in 250 ml of chloroform cooled to 0°. The mixture was stirred for 5 hr at 0°,