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Evaluation of Different Drugs in Two Models of Immediate Hypersensitivity

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Abstract—The effect of the calcium antagonists, verapamil, nicardipine and diltiazem, the two cromones, disodium cromoglycate and SM-857 (11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-2-carboxylic acid), and the anthelmintic, diethylcarbamazine citrate, have been compared on the ovalbumin (OA)-induced contraction of the isolated trachea and longitudinal muscle-myenteric plexus (LM-MP) from sensitized guinea-pigs. The calcium antagonists prevented the OA-induced contractions in LM-MP and to a lesser degree the OA-induced contractions in trachea. Similar doses of SM-857 protected both tissues but neither cromoglycate (10^{-5}M) nor diethylcarbamazine (10^{-5}M) affected these contractions. The OA-induced contraction in trachea had a tonic phase that was not present in the LM-MP response. Only the calcium antagonists succeeded in relaxing this OA tonic component, diltiazem being the more potent. These results unmask different mechanisms of drug action on immediate hypersensitivity and specific sensibilities, depending on the kind of tissue.

The clinical manifestations of allergy and anaphylactic reactions are produced by the release of chemical mediators from tissue mast cells and circulating basophil leucocytes (Sammuelsson 1983). These mediators are either preformed and stored within the cell in association with characteristic secretory granules (histamine, chemotactic factors, heparin, etc.) or are generated afresh by the oxidative metabolism of membrane-derived lipid components (leukotrienes, prostaglandins and thromboxanes). In total, these spasmogenic vasoactive and chemotactic factors act on distinct effector cells to induce the immediate and late phase reactions characteristic of asthma and other inflammatory responses.

As in other secretory processes, the primary event appears to be a rise in the intracellular concentrations of free calcium ions following cell activation. That an increase in free calcium itself is sufficient for secretion, has been clearly demonstrated by experiments in which exocytosis and mediator release have been induced by direct introduction of the cation into the cell, either by microinjection (Kanno et al 1973) or by means of calcium ionophores (Foreman 1981).

A variety of pharmacological agents are able to inhibit secretion in mast cells, depending largely on the cell type and experimental conditions adopted.

Given the essential nature of stimulus secretion coupling, it is natural that attempts have been made to account for the action of inhibitory compounds in terms of their effects on calcium homeostasis. On the other hand there is a wide variation in the behaviour and properties of different types of smooth muscles. The extent and significance of these differences are not clear.

To attempt to compare these differences in the anaphylactic responses, we have studied the effects of calcium antagonists (verapamil, nicardipine and diltiazem), two cromones with a mechanism of action not well known (sodium cromoglycate and SM-857L) and a lipoxygenase inhibitor (diethylcarbamazine) (Johnson et al 1984), on antigen-induced contraction of isolated trachea and ileal longitudinal smooth muscle from sensitized guinea-pigs.

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Material and Methods

Guinea-pigs (350–750 g) of either sex, were actively sensitized using ovalbumin (OA) (100 mg subcutaneous and 100 mg intraperitoneal). Three weeks later they were killed by stunning and bleeding.

Trachea smooth-muscle strips were prepared as previously described by Akcasu (1959). Trachea were excised from the animals, cleaned of adhering fat and connective tissue, and opened by cutting longitudinally opposite the trachealis cartilage. At the same time, a 20 cm portion of terminal ileum was removed and washed with Krebs solution at room temperature (ca 20°C). Longitudinal muscle-myenteric plexus (LM-MP) preparations were prepared from 4 cm segments as described by Paton & Zar (1968).

Organ bath experiments

Muscle strips were placed in 40 mL organ baths containing Krebs-Henseleit solution. They were attached by one end to a strain gauge for recording isometric tension. Initially a passive tension of 1 g in the LM-MP and 2 g in the trachea was used.

Experiments were begun after a period of equilibration (30 min for LM-MP and 1 h for trachea). The isometric contractions of the muscles were registered in a Panlab force transducer coupled with a Panlab polygraph. Immunospecific anaphylaxis was induced with ovalbumin, at a final concentration of 0·5 mg mL^{-1} in the LM-MP organ bath and 0·125 mg mL^{-1} in the trachea organ bath. These doses produced the maximum muscle responses.

In other trials the test drugs were added to the bath 10 min before the addition of maximal doses of ovalbumin, and, with some trachea strips, after the induction of control OA responses.

Statistical analysis

When the antigen response was induced in the presence of the test drugs, the per cent inhibition of the height of the contractile response was estimated against the assessed 100% value obtained when OA was added to a control preparation from the same animal. We compared this inhibition with the difference in the height between the responses when OA was added to two different control preparations from the same animal (control difference).

Data are expressed as mean % inhibition of isometric tension responses to ovalbumin ± standard error of the mean (s.e.m.); (n) represents the number of observations.

Tests of significance between the means of control values and those obtained in the presence of drugs were determined using Student's *t*-test for unpaired data.

Drugs

The drugs used were: diethylcarbamazine citrate, SM-857 (11-oxo-11*H*-pyrido[2,1-*b*]quinazoline-2-carboxylic acid), sodium cromoglycate, nicardipine HCl, verapamil HCl and diltiazem HCl.

Results

Effect of OA in the LM-MP and isolated trachea from sensitized guinea-pigs

OA elicited a concentration-related contraction of both muscles and the maximum was attained at 0·5 mg mL^{-1} in LM-MP and at 0·125 mg mL^{-1} in trachea. When OA maximal doses were used, both tissues became insensitive to further addition of OA. The OA-induced contraction of LM-MP was seen as a phasic component consisting of a sharp rise in tension followed by relaxation (Fig. 1A). The contractions of trachea also had a phasic component but it was followed by a tonic component still visible 1 h later (see Fig. 2A). The removal of OA from the medium caused a slow recovery of basal tone.

Effects of drugs on the OA-induced contraction of sensitized guinea-pig LM-MP

Neither diethylcarbamazine 10^{-5}M nor cromoglycate 10^{-5}M prevented in a significant way the OA-induced contraction in this tissue. On the other hand SM-857 and calcium antagonists showed an inhibitory dose-related activity. The highest blocking potency was observed with nicardipine. In contrast, SM-857, verapamil and diltiazem showed less activity, and much higher concentrations were necessary to obtain preventive effects with these drugs. Table 1 summarizes these data and Fig. 1 shows the effect of nicardipine, the most potent in preventing the OA-induced contraction in the LM-MP.

Effects of drugs on OA-induced contraction of sensitized guinea-pig isolated trachea

Neither diethylcarbamazine 10^{-5}M nor cromoglycate 10^{-5}M modified significantly the OA-induced contraction in this tissue. As with LM-MP, a concentration-related inhibition of the OA-induced contraction was observed with the other drugs. Nevertheless, contrary to the LM-MP results, calcium antagonists prevented the contractions at higher concentrations than did SM-857. Nicardipine 10^{-5}M and diltiazem 10^{-5}M significantly blocked the OA-induced contractions; verapamil 10^{-5}M also prevented the contractions, but differences were not statistically significant with this drug.

Table 2 summarizes these data and Fig. 2 shows the effect of SM-857, the more potent drug in preventing OA-induced contraction in the trachea. On the other hand, diethylcarbamazine 10^{-5}M sodium cromoglycate 10^{-5}M and SM-857 10^{-5}M failed to relax the trachea contracted by OA. Nevertheless when calcium antagonists were added to the organ bath the tissue reduced OA-induced contraction to a different degree. The reversion by nicardipine to 10^{-5}M was 37·6 ± 5·3% (6) and the reversion by verapamil 10^{-5}M was 41·5 ± 8·9 (6). When diltiazem 10^{-6}M was added the reversion was 34 ± 4·3% (6) but the tissue slowly recovered the basal tone when a 10^{-5}M concentration of this drug was used (see Fig. 3).

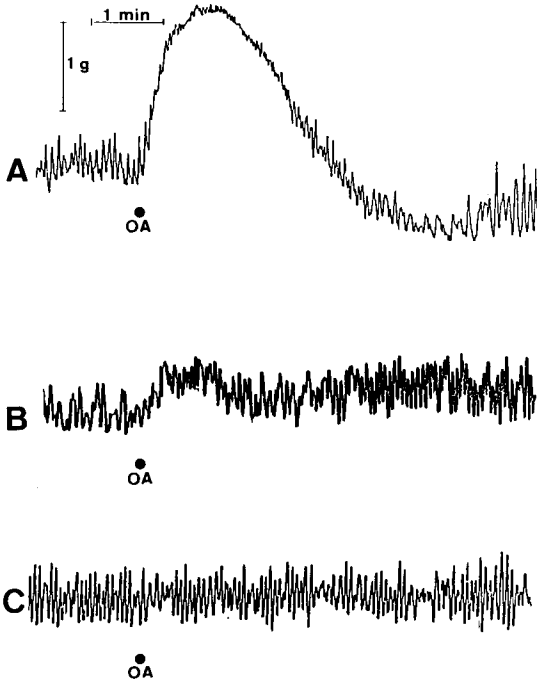

FIG. 1. Ovalbumin (OA)-induced contractions in sensitized guinea-pig LM-MP. A) Control. B) In the presence of nicardipine 10^{-8}M. C) In the presence of nicardipine 10^{-7}M.

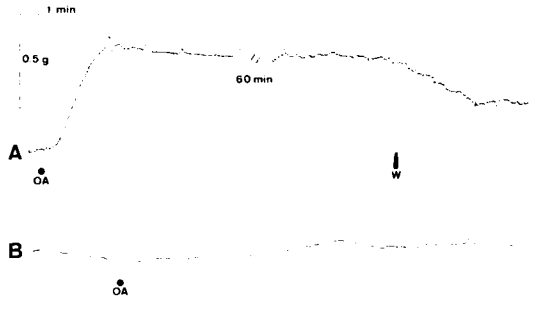

FIG. 2. Ovalbumin (OA)-induced contractions in sensitized guinea-pig trachea. A) Control (W) OA was removed by washing 1 h later. B) In the presence of SM-857 10^{-8}M.

Table 1. Percentage of the OA-induced contraction prevented by different drugs in the sensitized guinea-pig LM-MP.

Concn (M)	Nicardipine	Diltiazem	SM-857	Verapamil
5×10^{-9}	20·2 ± 7·4 (6)			
10^{-8}	71 ± 12·9 (6)*			
5×10^{-8}	90·8 ± 6·4 (6)***			
10^{-7}		54·3 ± 5·0 (6)*	46·3 ± 2·9 (6)	
5×10^{-7}		63·7 ± 4·7 (6)**	63·8 ± 7·2 (8)**	65·0 ± 7·7 (7)**
10^{-6}		87·8 ± 5·7 (6)***	72·1 ± 11·5 (7)**	70·8 ± 5·7 (7)**
5×10^{-6}				95·0 ± 3 (6)***

Each value represent mean ± s.e.m.; (n) number of test performed.
Significant differences * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Percentage of the OA-induced contraction prevented by different drugs in the sensitized guinea-pig trachea.

Concn (M)	SM-857	Nicardipine	Diltiazem	Verapamil
10^{-8}	43·5 ± 11·7 (6)			
10^{-7}	63 ± 10·3 (6)*			
10^{-6}	76 ± 11·7 (6)**	23·2 ± 7·8 (6)	46·2 ± 11·4 (6)	
5×10^{-6}		26·1 ± 8·8 (6)	54·5 ± 9·8 (6)	
10^{-5}		64·6 ± 5·7 (8)**	77 ± 11·2 (7)**	8·8 ± 5·6 (7)

Each value represent mean ± s.e.m.; (n) number of test performed.
Significant differences * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

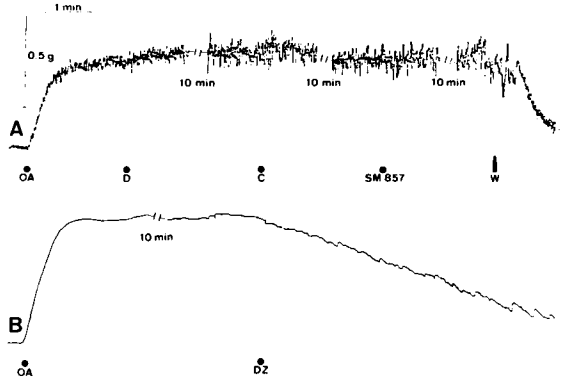

FIG. 3. Ovalbumin (OA)-induced contractions in sensitized guinea-pig trachea. A) A 10^{-5}M of different drugs were successively added at the contraction tissue: (D) diethylcarbamazine, (C) sodium cromoglycate and SM-857, (W) OA was removed by washing. B) The addition of diltiazem 10^{-5}M (DZ) relaxes the contracted tissue.

Discussion

The OA-induced contraction of sensitized guinea-pig tracheal muscle, showed a tonic phase that was not evident in longitudinal ileal muscle. Since the tonic phase depends on internal calcium stores (Bolger et al 1983), such a difference suggests that calcium stores in the ileum are less important than in the trachea and they are completely emptied in the LM-MP when the stimulus is applied.

Neither sodium cromoglycate 10^{-5}M nor diethylcarbamazine 10^{-5}M showed any activity on the contractile response to OA on either muscle. However, cromoglycate has been shown to inhibit the release of histamine from peritoneal mast cells but was without effect on mucosa mast cells (Schwender 1983), and diethylcarbamazine has been found to inhibit antigen-induced release of both histamine and slow-reacting substances of anaphylaxis from human lung fragments (Abaitey & Parratt 1977). Moreover Mallen et al (1965) have shown diethylcarbamazine to be effective in the management of bronchial asthma. Therefore, the results appear to depend on the cell source, species, nature, and the concentration of the contractile stimulus, as well as on the concentration of the drugs used.

On the other hand, SM-857 and calcium antagonists showed a dose-related inhibitory effect on the OA-induced contraction of the LM-MP and the trachea. Except for SM-857, mode of action of which is unknown (Investigational Brochure SM-857, Boehringer Ingelheim Ltd, 1980), these data suggest a dose-related inhibitory effect on calcium uptake, since the OA-induced contraction is a calcium-dependent phenomenon (Kirkpatrick 1975; Foreman et al 1977; Ishizaka & Conrad 1983). Malagodi & Chion (1974) reported similar results on calcium uptake of sensitized mast cells after an antigen challenge with nifedipine, a drug belonging to the dihydropyridines, as does nicardipine.

The current study has demonstrated that the in-vitro calcium entry blockers examined have an effect on the OA-contractile response of sensitized guinea-pig ileal longitudinal muscle, but they are less active in preventing the OA-contraction of the isolated tracheal muscle. This may be explained by the differences in tissue sensitivity. The results in the LM-MP preparation indicate that the influx of extracellular calcium has function in the contractile activity of this tissue. Similarly, Chang & Triggle (1982) also reported a role for extracellular calcium in the excitation-contraction coupling process of the guinea-pig ileum in response to

muscarinic agents and to potassium. Verapamil and diltiazem were less effective in preventing the OA-induced contraction in the LM-MP preparation and verapamil showed no significant prevention of the OA-response of the trachea. On the other hand, nicardipine was the most active calcium antagonist in the LM-MP. Therefore, since these drugs share the same mode of action, the effectiveness of each is quite different. The findings in the LM-MP with calcium antagonists suggest that these drugs may be useful in clinical disorders in which an immediate hypersensitivity reaction is thought to be involved. Even though they are weak in blocking the OA-induced contraction in the tracheal smooth muscle, all of them showed a direct relaxing activity on that tissue. Other authors have also demonstrated similar effects on respiratory smooth muscle in-vitro by diltiazem (Nagao et al 1981), by verapamil (Eberlin et al 1982; Weiss et al 1982) and by nifedipine (Drazen et al 1983). The contractile activity of respiratory smooth muscle depends on the intracellular calcium provision (Coburn 1977). That, in part, is regulated by internal calcium stores and in part by calcium influx from the external medium. The percentage from both sources depends on the tissue, the contractile stimulus and the contractile response (Meisheri et al 1981). Himori & Taira (1980) showed in a direct comparison between cardiovascular tissue and airway tissue, that verapamil and nifedipine were clearly more effective on vascular tissue than on airway tissue. If the physiology of calcium in airway smooth muscle were fully understood, it may be possible to design a more specific calcium antagonist that would preferentially act on airway smooth muscle. Likewise, in those patients with ischaemic heart disease or hypertension and airway obstruction, in whom beta-blocking drugs are contraindicated, calcium blockers are useful.

Diethylcarbamazine and sodium cromoglycate did not relax the tracheal smooth muscle and that is consistent with their inability to reverse the bronchial spasm in clinical practice. The cromone SM-857 was relatively effective in preventing the OA-induced contraction of the trachea but it was not active as a relaxant of antigen-induced contraction. The mechanism of action of this drug has to be studied further but these results raise the possibility that mast cell degranulation is involved.

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