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Inhibition of allergic and non-allergic histamine secretion from rat peritoneal mast cells by calcium antagonists

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1 Bepridil, TMB-8 (8-(diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride),diltiazem, verapamil and nifedipine exerted concentration-dependent inhibition of antigen and calcium ionophore A23187-induced histamine release from rat peritoneal mast cells.

2 The inhibitory effects of verapamil and bepridil against calcium ionophore A23187-induced histamine secretion were antagonized by increased Ca^{2+} concentrations in the extracellular medium. These observations suggest that both agents act by interfering with the influx of Ca^{2+} into the mast cells.

3 The inhibitory activities of five different Ca^{2+} channel blockers on allergic and non-allergic histamine secretion from rat peritoneal mast cells varied considerably depending upon the nature of the secretagogue as well as concentration and type of Ca^{2+}-antagonist examined.

Introduction

Calcium plays a critical role in the synthesis and release of chemical mediators from leukocytes, mast cells, and other cells (Foreman, 1981; Middleton, 1983). Recently, 'calcium entry blockers' have been used to explore the role of Ca^{2+} in the pathophysiology of immediate hypersensitivity reactions. These substances have been shown to inhibit the synthesis and release of slow reacting substance of anaphylaxis (SRS-A, leukotrienes), platelet activating factor (Paf-acether) and thromboxane A_2 (Cerrina *et al*, 1982; Srivastava & Awasthi, 1983; Lee *et al*, 1983; Jouvin-Marche *et al*, 1983). Nifedipine also inhibits the release of lysosomal enzymes from human neutrophils (Jouvin-Marche *et al.*, 1983). They also have been reported to exert highly variable effects on allergic and non-allergic histamine release from rat peritoneal mast cells and human basophils (Diamant & Patkar, 1975; Atkins *et al.*, 1981; Norn *et al.*, 1982; Bedard & Busse, 1983; Sierchio *et al.*, 1983). In this investigation, the ability of the calcium entry blocker, bepridil (a new anti-anginal agent) to influence antigen and calcium ionophore A23187-induced histamine secretion from rat peritoneal mast cells was studied and compared with the selected reference agents.

Methods

Adult male Sprague-Dawley rats weighing between 300–500 g were sensitized with ovalbumin (OA) (1 mg, i.p.) and killed *Bordetella pertussis* 2.2×10^{10} organisms (i.p.) (Mota, 1964). Two to three weeks later, peritoneal mast cells were harvested.

The remainder of the methods for the isolation of peritoneal mast cells in Tris-gel buffer and for fluorometric assay of histamine in the presence and absence of drugs was similar to that described earlier (Chand *et al.*, 1983a,b), except Tris-gel CM buffer was used for ovalbumin ($10\,\mu g\,mg^{-1}$) plus phosphatidylserine ($10\,\mu g\,ml^{-1}$)-induced histamine release. The composition (mM) of Tris-gel CM was: Tris 25.0, NaCl 120.0, KCl 5.0, $CaCl_2$ 0.6, $MgCl_2$ 1.0 and 0.1% gelatin (pH 7.2) at 37°C. When calcium ionophore A23187 was used as a secretagogue, Tris-gel C buffer was used, and peritoneal mast cells were obtained from nonsensitized rats. (Chand *et al.*, 1983a).

In order to determine whether the inhibitory activity of varapamil and bepridil on A23187 ($0.2\,\mu M$)-stimulated histamine release was associated with the inhibition of Ca^{2+} influx, additional histamine re-

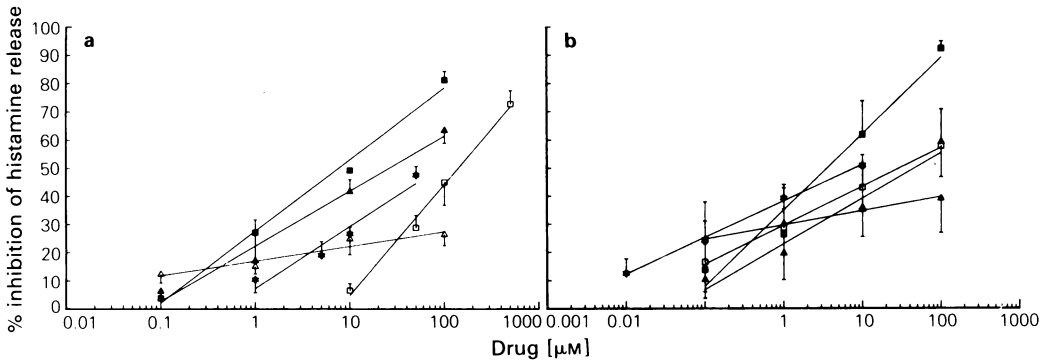

Figure 1 Concentration-effect (inhibition) curves of bepridil (★), TMB-8 (■), diltiazem (▲), nifedipine (△) and verapamil (□) on antigen ($10\,\mu g\,ml^{-1}$ ovalbumin + $10\,\mu g\,ml^{-1}$ phosphatidylserine) (a)- and calcium ionophore A23187 (0.2 μM) (b)-induced histamine release from rat peritoneal mast cells. Mean % histamine release induced by antigen and A23187 from control cell suspensions varied between 39.8 to 71 and 66 to 75.6, respectively. Each point represents mean of 4 to 6 experiments; s.e.mean shown by vertical lines. Cells were preincubated with the drugs for a period of 10 min at 37°C before the addition of secretagogue. Release reaction was continued for an additional 30 min. Spontaneous histamine release occurring in the absence of drugs and secretagogues was always less than 1%.

lease experiments were performed in Tris-gel C buffer containing 0.6 mM and 3.0 mM Ca^{2+} concentrations on the same cell suspensions.

Results

Bepridil, TMB-8 (8-(diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride), verapamil, diltiazem and nifedipine exerted concentration-dependent inhibition of antigen- and calcium ionophore A23187-induced histamine secretion from peritoneal mast cells (Figure 1a, b). Nifedipine in a concentration range of 0.1–100.0 μM produced a concentration-dependent 12–26% inhibition of allergic histamine release (Figure 1a). Diltiazem in a concentration range of 0.1–100.0 μM produced only 24–39% inhibition of ionophore-induced histamine secretion (Figure 1b).

The IC_{50s} of Ca^{2+} antagonists against allergic and non-allergic histamine release from rat peritoneal mast cells have been summarized in Table 1.

Effect of buffer calcium concentration

The concentration-effect (inhibition) curve of verapamil and bepridil against A23187-induced histamine release in Tris-gel C buffer (Ca^{2+} = 0.6 mM) was shifted to the right by high Ca^{2+} concentrations (3.0 mM) in the buffer (Figure 2a, b).

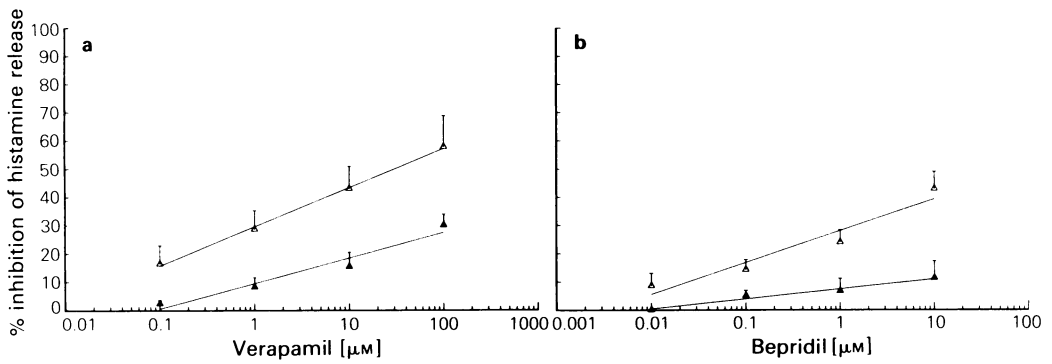

Figure 2 Concentration-effect (inhibition) curves of verapamil (a) and bepridil (b) against calcium ionophore A23187 (0.2 μM)-induced histamine secretion from rat peritoneal mast cells in Tris-gel buffer containing 0.6 M (△) and 3.0 mM (▲) Ca^{2+}. Mean % control histamine release was 75.6 to 86.6 and 70.8 to 71.8 in the presence of 0.6 and 3.0 mM Ca^{2+} ion concentrations, respectively. Each point represents mean of 5 experiments, s.e.mean shown by vertical lines.

Table 1 Summary of the inhibitory activities (IC_{50s}) of calcium antagonists on allergic and non allergic histamine release from rat peritoneal mast cells

	IC_{50}, (μM) (95% confidence limits)	
Drug	OA (10 μg ml^{-1})+ PS (10 μg ml^{-1})	A23187 (0.1 μg ml^{-1})
Bepridil	88.1 (est.) (35.7 – 217.3) Slope = 21.97 I = 7.21 *r* = 0.95	8.1 (1.7 – 39.2) Slope = 12.97 I = 38.19 *r* = 1.00
TMB-8	7.6 (5.6 – 10.2) Slope = 25.45 I = 27.58 *r* = 0.99	3.6 (1.7 – 7.6) Slope = 29.07 I = 34.95 *r* = 0.97
Diltiazem	25.99 (13.5 – 50.1) Slope = 19.60 I = 22.24 *r* = 0.98	>100 Slope = 5.07 I = 29.65 *r* = 0.98
Nifedipine	>100 Slope = 5.17 I = 16.97 *r* = 0.92	46.7 (5.6 – 386.7) Slope = 16.25 I = 22.88 *r* = 0.96
Verapamil	140.1 (95.6 – 205.3) Slope = 39.43 I = 0 *r* = 0.99	30 (4.3 – 208.3) Slope = 13.84 I = 29.52 *r* = 1.00

I = Intercept (calculated % inhibition of histimine release by drugs at 1 μM concentration)
OA = Ovalbumin; PS = Phosphatidylserine

Discussion

In this study, calcium entry blockers exerted concentration-dependent inhibition of allergic and non-allergic histamine release from rat peritoneal mast cells. In contrast to these observations, Sierchio *et al.* (1983) reported that verapamil, nifedipine, diltiazem and flunarizine exert little or no inhibition of histamine release by compound 48/80 (100 μM), A23187 (2 μM) and antigen. Similarly, Atkins *et al.* (1981) reported little or no effect of verapamil, methoxyverapamil (D-600), nifedipine and TMB-8 on allergic histamine release from human basophils, an observation which suggests that the Ca^{2+} channels used in allergic histamine secretion from human basophils are different and are probably insensitive to the calcium entry blockers. Bedard & Busse (1983), however, showed a concentration-dependent inhibition of A23187-stimulated histamine secretion from human basophils by nifedipine. Cerrina *et al.* (1982) showed that nifedipine (0.5 to 10 μM) produces a concentration-dependent inhibition of allergic release of SRS-A from human lung tissue but produces little or no effect on allergic histamine secretion. They concluded that nifedipine is capable of interfering with the synthesis and release of newly formed chemical mediators only. The differences in the inhibitory activities of calcium entry blockers suggest the existence of heterogeneity of Ca^{2+} channels involved in the influx of Ca^{2+} in response to allergic and non-allergic stimuli in different target cells of allergic inflammation, e.g., human mast cells, basophils, and rat mast cells.

The highly variable effects of Ca^{2+}-entry blockers on allergic and non-allergic histamine secretion

(Table 1) may be related to the differences in their lipid/water solubilities, ability to accumulate inside the cells as well as their calmodulin-inhibiting activities (Pang & Sperelakis, 1984; Lugnier *et al.*, 1984). Bepridil is a potent calmodulin inhibitor (Lugnier *et al.*, 1984). TMB-8 is an intracellular calcium antagonist. Therefore, in addition to the blockade of Ca^{2+} influx in the mast cells, Ca^{2+}-entry blockers could also influence cellular or intracellular Ca^{2+}-dependent steps in histamine secretion.

The antagonism of the inhibitory effects of bepridil and verapamil against A23187-stimulated histamine secretion from rat peritoneal mast cells by increased Ca^{2+} (3.0 mM) concentrations in the buffer seem to suggest that both drugs are capable of interfering with the influx (uptake) of Ca^{2+} in the mast cells (basophils). The exact site of action of calcium entry blockers in obstructive respiratory diseases is not yet known. It remains to be established whether different calcium entry blockers exert differential inhibitory effects on Ca^{2+}-dependent activation of calmodulin, phospholipase A_2 and 5-lipoxygenase in mast cells and basophils that may account for the differences in their inhibitory activities on histamine secretion in response to diverse secretagogues.

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