Inhibition of histamine release from dispersed human lung and tonsillar mast cells by nicardipine and nifedipine

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1 Calcium entry blocking drugs attenuate antigen-induced bronchoconstriction in asthma which is mast cell mediated. We have investigated the effects of two calcium uptake blockers, nicardipine and nifedipine on histamine secretion from human mast cells dispersed from lung and tonsillar tissue. Mast cells were activated for secretion with anti-human IgE or calcium ionophore, A23187.

2 Nicardipine and nifedipine caused a concentration-related inhibition of IgE-dependent histamine release from both lung (IC₃₀ 10 µM and 4.4 µM) and tonsillar (IC₃₀ 21 µM and 47 µM) mast cells. Nicardipine and nifedipine also inhibited mast cell histamine release induced by A23187 with IC₃₀ values of 14 µM and 67 µM for lung and 15 µM and 30 µM for tonsillar mast cells.

3 In the absence of drugs, increasing the extracellular calcium concentrations from 0.2 to 5 mM caused a concentration related increase in IgE-dependent histamine release from tonsillar mast cells. Both nicardipine and nifedipine (50 µM) displaced the concentration-effect curve to the right.

4 Nicardipine (0.01–100 µM) caused a concentration related inhibition of rat kidney histamine methyltransferase activity used in the radioenzymatic assay of histamine (*k_i* of 7.5–12 µM) whereas nifedipine was only a weak inhibitor. Nicardipine also interfered with the spectrofluorimetric assay after exposure to ultraviolet light.

5 These observations demonstrate that nicardipine and nifedipine inhibit IgE-dependent and ionophore stimulated mediator secretion from human mast cells. The lack of stimulus-related specificity and the high drug concentrations required suggest that classical calcium channel blockade is not responsible for inhibition of mast cell mediator release. Furthermore, we suggest that inhibition of mast cell mediator release is unlikely to be the mechanism by which these drugs alleviate asthma.

Keywords calcium entry blockers nicardipine nifedipine mast cells asthma

Introduction

Stimulation of many contractile and secretory cells results in their membranes becoming transiently permeable to extracellular calcium ions which then enter the cells down a concentration

gradient. The consequential elevation of free intracellular calcium is necessary for activation of calcium-dependent enzymes involved in stimulus-contraction or stimulus-secretion

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coupling. In asthma, calcium-dependent processes which may be involved in causing airways obstruction include contraction of airways smooth muscle, secretion of preformed and newly generated mediators from mast cells and other inflammatory cells, stimulation of parasympathetic neurohumoral transmission and mucus secretion (Middleton, 1980; Church *et al.*, 1982; Barnes, 1983; Fanta & Drazen, 1983; Middleton, 1983). Thus, agents which have the capacity to reduce the stimulus-associated cellular influx of calcium ions may theoretically be of benefit in the treatment of asthma.

Clinically, calcium entry blockers have been shown to protect against bronchoconstriction in asthma induced by exercise (Cerrina *et al.*, 1981; Barnes *et al.*, 1981; Patel, 1981a, b) and by inhalation of antigen (Henderson *et al.*, 1983a). Most studies have shown little or no effect of these drugs on resting airways calibre. However, one placebo controlled study in which patients with asthma were treated with verapamil has reported bronchodilation and a reduction in diurnal bronchoconstriction (Patakas *et al.*, 1983).

The mechanisms underlying the protective effect of these drugs in asthma are not yet understood. The inhibitory effects of verapamil and nifedipine against bronchoconstriction induced by histamine (Williams *et al.*, 1981; Malik *et al.*, 1982; Corris *et al.*, 1983), methacholine (Malik *et al.*, 1982) and hyperventilation (Rolla *et al.*, 1982; Henderson *et al.*, 1983b) suggest a direct action on airways smooth muscle. In support of this view, bronchoconstriction in the dog induced by inhaled citric acid and methacholine is attenuated by nifedipine (Brugman *et al.*, 1983) and calcium antagonists reverse contraction of human and animal airways smooth muscle preparations *in vitro* (Butchers *et al.*, 1982; Fanta *et al.*, 1982; Henderson *et al.*, 1983a). Another possible mechanism of action for calcium antagonists in asthma is inhibition of calcium-dependent mediator secretion from mast cells and other inflammatory cells in the bronchi. *In vivo*, sublingual nifedipine reduces the rise in plasma levels of histamine observed following exercise provocation in patients with asthma (Barnes *et al.*, 1981). *In vitro*, calcium entry blockers reduce histamine release from rat mast cells induced by a variety of stimuli (Suzuki *et al.*, 1982; Tanizaki *et al.*, 1983) and from human basophil leucocytes (Jensen *et al.*, 1983). In human lung fragments, the IgE-dependent generation and release of SRS-A leukotrienes is more effectively inhibited than is the release of preformed mediators such as histamine (Butchers *et al.*, 1982; Cerrina *et al.*, 1982; Lee *et al.*, 1983).

To investigate further the pharmacological

effects of calcium entry blockers, we have studied the effects of two dihydropyridine derivatives, nifedipine and nifedipine, on histamine release from dispersed human lung and tonsillar mast cells induced immunologically by challenge with anti-human IgE and non-specifically by incubation with calcium ionophore A23187. Lung mast cells were used because of their obvious association with asthma. Because of the increasing evidence of mast cell heterogeneity within species, comparisons were made with the tonsillar mast cells, a lymphoid derived mast cell.

Methods

Dispersal and challenge of human mast cells

Macroscopically normal human lung tissue obtained from surgically resected specimens and surgically removed human tonsils were chopped finely with scissors and digested for 30 min at 37°C with pronase (2 mg/ml) and chymopapain (0.5 mg/ml) in 20 mM HEPES buffered salt solution at pH 7.4. The dispersed cells were separated from tissue fragments by sequential sieving through 800 µm and 60 µm nylon gauze (Church *et al.*, 1982, 1983). Staining of wet preparations with Kimura's stain showed mast cells to comprise 1 to 3% of total nucleated cells from lung and 0.03 to 0.1% from tonsils.

Before assessment of histamine release, tonsillar mast cells were passively sensitized by incubation for 15 min at 37°C with 1 ml of serum from an atopic donor sensitive to grass pollens. The lung mast cells were not passively sensitized. Duplicate aliquots containing 10⁵ mast cells in 10 mM HEPES buffered salt solution at pH 7.4 containing 0.02% human serum albumin (HBSS) were preincubated at 37°C for 10 min before challenge in the absence or presence of nifedipine or nifedipine. Cells were challenged by incubation with goat anti-human IgE (1/300 dilution) or calcium ionophore A23187 (0.3 µM) for 15 min at 37°C in a final volume of 1 ml. Release reactions were stopped by centrifugation at 250 g for 10 min at 4°C and the cell pellets and supernatants separated.

To study the effects of extracellular calcium concentrations on drug activity, 50 µl of cell suspensions containing 10⁵ mast cells were added to 900 µl of HBSS of appropriate calcium and drug concentrations and incubated for 10 min at 37°C. The cells were challenged by addition of 50 µl anti-IgE or A23187 solution and incubation for a further 15 min.

All incubations were performed under sodium light (Gallenkamp LCH-600-Q) in a dark room

to prevent photochemical decomposition of the dihydropyridines.

Histamine assays

Histamine was assayed either by automated spectrofluorimetry or by a sensitive radio-transfer assay (Church *et al.*, 1982). For spectrofluorimetric assay, cell supernatants were acidified to 5% with trichloroacetic acid (TCA). Total histamine was measured in duplicate samples of untreated cells disintegrated with 5% TCA. For the radiotransfer assay, supernatants were not acidified but the cells disintegrated by freezing at -20°C and thawing. All samples were stored at -20°C until assay. Net histamine release is expressed as the percentage of total histamine released into the supernatant corrected for spontaneous release.

Before the histamine assays were used to assess mast cell mediator release, the effects of nicardipine and nifedipine on standard concentrations of histamine were examined. The results of these experiments are described later.

Statistical analysis

Drug effects were analysed by comparing net histamine releases of control and treated cells using Student's *t*-test for paired data. IC_{30} values (concentrations of drugs calculated to inhibit histamine release from 30%) were calculated from best fit linear regression lines. Results are expressed as mean $\pm$ s.e. mean.

Materials

Nicardipine and nifedipine were donated by Dr D. Parrott of Syntex Research and Dr K. D. Young of Bayer UK, respectively. They were dissolved in dimethylsulphoxide (DMSO, Sigma) and diluted with HBSS to a final DMSO concentration of 0.5% which alone did not affect histamine release.

Goat anti-human IgE was heat inactivated by incubation for 1 h at 56°C . Calcium ionophore A23187, human serum albumin (factor V), deoxyribonuclease (DNase, bovine pancreas), chymopapain (papaya latex), pronase (Streptomyces griseus) and *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES) were all obtained from Sigma. Trichloroacetic acid and other chemicals were obtained from BDH Chemicals. The composition of the 10 mM HEPES buffered salt solution (HBSS) was: NaCl 137 mM, KCl 2.7 mM, NaH_2PO_4 0.4 mM, MgCl_2 0.5 mM, CaCl_2 0.9 mM, glucose 5.5 mM and HEPES 10 mM. The HBSS was adjusted to pH 7.4 with 2 M NaOH and 0.2 mg ml^{-1} human

serum albumin was added.

Results

Effect of nicardipine and nifedipine on mast cell histamine release

Both nicardipine and nifedipine (0.01 to 100 μM) caused concentration-related inhibition of histamine release from dispersed human lung and tonsillar mast cells induced by a 1/300 dilution of anti-IgE (Figure 1). In lung mast cells, in which anti-IgE induced a $10.2 \pm 2.5\%$ net histamine release, only drug concentrations above 1 μM caused significant ($P < 0.05$) inhibition. The IC_{30} values for nicardipine and nifedipine were 4.4 μM and 10 μM respectively. In tonsillar mast cells, in which the net anti-IgE induced histamine release was $15.3 \pm 3.0\%$, only drug concentrations above 10 μM caused significant inhibition. The IC_{30} values for nicardipine and nifedipine in these cells were 21 μM and 47 μM respectively. The differences between IC_{30} values obtained in lung and tonsillar mast cells were not statistically significant.

Nicardipine and nifedipine also inhibited histamine release induced by calcium ionophore A23187, nicardipine again being the more potent than nifedipine (Figure 2). In lung mast cells, in which A23187 induced $35.6 \pm 5.4\%$ net histamine release, the IC_{30} values for nicardipine and nifedipine were 14 μM and 67 μM respectively. In tonsillar mast cells in which A23187 induced release was $35.1 \pm 5.0\%$, the respective IC_{30} values were 15 μM and 30 μM .

Experiments were performed to determine the influence of varying the extracellular calcium concentration on the inhibitory properties of nicardipine and nifedipine. In the absence of drugs, increasing the extracellular calcium concentration from 0.2 to 5 mM resulted in a concentration-related increase in the IgE-dependent histamine release from tonsillar mast cells from 11.8% to 30.9%. Nifedipine 50 μM (Figure 3) caused a parallel displacement of this line to the right, suggesting that increased extracellular calcium does not overcome the inhibitory effects of nifedipine. Similar results were obtained with nicardipine.

Effects of nicardipine and nifedipine on histamine assays

When protected from light neither nicardipine nor nifedipine at 100 μM interfered with the spectrofluorimetric assay of 200 ng/ml histamine standards. Exposure of nicardipine to ultraviolet light (365 nm) for up to 30 min resulted in the

Figure 1 Inhibition of anti-IgE induced histamine release from (a) human lung mast cells and (b) human tonsillar mast cells by nicardipine (○) and nifedipine (●). Cells were preincubated with the drugs for 10 min before challenge with a 1/300 dilution of anti-IgE. In lung mast cells spontaneous histamine release was $8.5 \pm 1.1\%$ and net anti-IgE induced release $10.2 \pm 2.5\%$ in five experiments. In tonsillar mast cells spontaneous release was $12.5 \pm 3.0\%$ and net anti-IgE induced release $15.3 \pm 3.0\%$ in four experiments. Net control histamine releases have been normalized to 100% for illustrative purposes.

Figure 2 Inhibition of ionophore A23187-induced histamine release from (a) human lung mast cells and (b) human tonsillar mast cells by nicardipine (○) and nifedipine (●). Cells were preincubated with the drugs for 10 min before challenge with $0.3 \mu\text{M}$ A23187. In lung mast cells spontaneous histamine release was $8.5 \pm 1.1\%$ and net A23187-induced release $35.6 \pm 5.4\%$ in five experiments. In tonsillar cells mast spontaneous release was $13.2 \pm 2.7\%$ and net A23187 induced release $35.1 \pm 5.0\%$ in four experiments. Net control histamine releases have been normalized to 100% for illustrative purposes.

Figure 3 Variation of extracellular calcium concentration as anti-IgE-induced histamine release from human tonsillar mast cells in the absence (▲) and presence (●) of 50 μ M nifedipine. Cells were preincubated with nifedipine for 10 min before challenge. Spontaneous histamine release in this experiment was 8.3%.

drug causing an exposure-related decrease in the sensitivity of the assay (Figure 4). After 30 min exposure, the apparent histamine concentration was only 35% of the true concentration. Ultra-violet irradiation of nifedipine had less effect, the slight exposure-related inhibition of the assay being non-significant.

In the methyltransferase assay, nicardipine (0.01–100 μ M) caused a concentration-related inhibition of S-adenosylmethionine dependent methylation of histamine, leading to an apparent reduction in the concentration of a standard concentration (5 ng/ml) of histamine (Figure 5). Under these conditions the IC_{50} for nicardipine was 1 μ M. To elucidate the type of enzyme inhibition, the rates of methylation of 0.25, 0.5 and 1 μ M histamine were determined in the presence of 1 to 100 μ M nicardipine in two experiments (Beaven & Shaff, 1978). Line-weaver-Burk and Dixon plots showed inhibition to be non-competitive. The k_i for nicardipine calculated from Dixon plots was between 7.5 and 12 μ M. In contrast nifedipine was only a weak inhibitor of the methyltransferase assay (Figure 5), causing a $21.5 \pm 11\%$ ($P < 0.05$) inhibition of the assay of 5 ng/ml histamine at 100 μ M.

The inhibitory effect of these dihydropyridine derivatives shown on histamine methyltransferase precluded the use of the radiotransfer assay for histamine in assessing the effect of

these drugs on mast cell histamine release. The studies on the modulation of mast cell histamine release by nicardipine and nifedipine employed to spectrofluorimetric assay performed in the dark to prevent photochemical interference.

Discussion

The dihydropyridine calcium entry blockers nicardipine and nifedipine both reduced histamine release from human dispersed lung and tonsillar mast cells stimulated with anti-IgE and calcium ionophore A23187. The concentrations required were in the range 4.4 to 47 μ M. These results accord with results in human basophils (Jensen *et al.*, 1983; Bedard & Busse, 1983) and rat mast cells (Tanizaki *et al.*, 1983) suggesting inhibition produced by calcium entry blockers is not subject to the wide species variation as has been observed with sodium cromoglycate and β -adrenoceptor stimulants (Church & Young, 1983).

Mast cells, like other secretory cells, require an influx of extracellular calcium in order to couple activation to their secretory process (Church *et al.*, 1982; Ishizaka, 1981; Pearce, 1982). At rest, extracellular calcium is of the order of 1 mM and free cytosolic calcium around 0.1 μ M, the transmembrane concentration difference of 10,000 being maintained by a Ca^{++}

Figure 4 Effects of 100 μM nicardipine (○) and 100 μM nifedipine (●) on the fluorimetric assay of histamine following exposure of the drugs to ultraviolet light at 365 nm. Drugs were added to duplicate 200 ng/ml standard concentrations of histamine immediately before assay in two experiments.

Figure 5 Effects of nicardipine (○) and nifedipine (●) on the methyltransferase assay of histamine. Drugs were added to triplicate 5 ng/ml standard concentrations of histamine immediately before assay in four experiments.

ATPase extrusion pump and a Na/Ca^{++} exchange mechanism (Cavero & Spedding, 1983). When stimulated, the cell membrane becomes transiently permeable to calcium, allowing the cytosolic calcium concentration to rise some 100-fold to around $10\text{ }\mu\text{M}$ (Cavero & Spedding, 1983). In smooth muscle, the opening of calcium channels may be voltage operated, associated with depolarization or the cell membrane, or receptor operated, involving little or no depolarization (Bolton, 1979). Calcium entry blockers are particularly effective against voltage operated channels but are markedly less effective against receptor operated channels. For example, contraction of rabbit aortic strips induced by stimulation of voltage operated channels are blocked by nifedipine at concentrations in the nanomolar range whereas those stimulated by receptor operated channels are poorly inhibited by concentrations as high as $10\text{ }\mu\text{M}$ (Towart, 1982).

Although the type of calcium channel in mast cells is unknown, the observation that inhibition of IgE-dependent histamine release requires nicardipine or nifedipine concentrations in the $10\text{ }\mu\text{M}$ range suggests that voltage operated channels are not involved. The findings that histamine secretion induced by calcium ionophore A23187, which bypasses physiological calcium channels by inserting calcium directly through the membrane (Pearce, 1982), is blocked by similar concentrations of inhibitors and that inhibition of IgE-dependent histamine secretion is not reversed by increasing the extracellular calcium concentration, indicate a pharmacological mechanism not involving regulation of calcium influx. As many non-specific pharmacological effects (including Na^+ , K^+ and Cl^- channel blockade, local anaesthetic activity membrane stabilization, α - and β -adrenoceptor blockade, antihistaminic and antimuscarinic activity, phosphodiesterase and calmodulin inhibition and inhibition of oxidative phosphorylation) have been ascribed to various calcium antagonists (Kazda *et al.*, 1983) the possible effects on mast cells are numerous. As phosphodiesterase inhibitors are known to inhibit mast cell mediator secretion, the observation by Nishikori *et al.* (1981) that nicardipine inhibits cyclic AMP phosphodiesterases with K_i values of 5.3 and $13.2\text{ }\mu\text{M}$ may have particular relevance.

Nicardipine, and to a lesser extent, nifedipine, were found to interfere with the assays of histamine. When protected from light, neither drug interfered with the spectrofluorimetric assay. However, exposure of nicardipine to ultraviolet light leads to the generation of a product which inhibited either the extraction or fluorescence of histamine. In contrast, nifedipine, which is reported to lose clinical activity on exposure to light, did not form a product which markedly interfered with the histamine assay.

Nicardipine also caused a non-competitive inhibition of rat kidney methyltransferase used in the enzymatic radiotransfer assay of histamine. Besides being the basis of the methyltransferase assay for histamine, this enzyme provides a major route for the inactivation of histamine *in vivo*. The K_i value of around $10\text{ }\mu\text{M}$ and the IC_{50} in the assay of $1\text{ }\mu\text{M}$ suggest an effect within the concentration range for inhibition of histamine release. The ability to inhibit histamine methylation, a property shared by many histamine H_1 - and H_2 -receptor antagonists, antimalarials and local anaesthetics (Thithapandha & Cohn, 1978; Beaven & Shaff, 1978) prohibits the use of this histamine assay with nicardipine and may have clinical implications with high doses of drug.

The crucial question to be answered from this study is the relevance of inhibition of mast cell histamine release in the protective action of calcium entry blockers in asthma. The finding that the concentrations in the $10\text{ }\mu\text{M}$ range required for inhibition of histamine release are in excess of peak plasma levels (0.05 to $0.5\text{ }\mu\text{M}$) achieved with conventional doses of nifedipine and nicardipine suggests that mast cell stabilization is unlikely to occur *in vivo*. However, Jensen *et al.* (1983) have reported a slight reduction of basophil histamine release following oral therapy with nifedipine. Furthermore, Butchers *et al.* (1981) and Lee *et al.* (1983) showed that the production of SRS-A leukotrienes from human lung fragments is more sensitive to blockade by calcium entry blockers than is the release of histamine. Nevertheless, the results of our study suggest that any protective effect of calcium entry blockers in asthma is more likely to result from actions on the bronchial musculature than inhibition of mast cell mediator release.

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