Effect of calcium antagonists on histamine release from human basophil leukocytes

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The effect of calcium antagonists on basophil activation was investigated in 14 subjects. Verapamil, methoxyverapamil and diltiazem, in a concentration range of 1–100 μM , exert a concentration-dependent inhibition on the human basophil histamine release induced by calcium ionophore A23187, zymosan activated human serum and grass pollen allergens. This inhibitory effect is highly variable from subject to subject and from drug to drug. At concentrations higher than 200 μM , verapamil, methoxyverapamil and diltiazem induce a calcium independent histamine secretion from basophil leukocytes. This mediator release is associated with an increase in LDH levels in the cell supernatant and seems to be due to a cytotoxic effect.

Keywords calcium antagonists basophil leukocytes histamine release

Introduction

The release of allergic mediators from mast-cells and basophils depends on transmembrane flow of calcium ions from the interstitial fluid into the cytoplasm (Kalinin & Austen, 1973).

Calcium antagonist drugs were shown to reduce synthesis and release of slow reacting substance of anaphylaxis (SRS-A) (Cerrina *et al.*, 1982). Controversial findings were reported about the effect of calcium antagonists on allergic and non allergic histamine release from rat peritoneal mast-cells and human basophils, with some authors (Jensen *et al.*, 1983; Chand *et al.*, 1984) describing an inhibitory activity and others (Atkins *et al.*, 1981; Sierchio *et al.*, 1983) observing little or no reduction in mediator release.

In previous experiments (Miadonna *et al.*, 1983) we observed that verapamil could inhibit *in vitro* basophil degranulation induced by grass pollen allergens. In the present study we have evaluated the *in vitro* effect of different concentrations of calcium antagonists (verapamil, methoxyverapamil, diltiazem) on histamine release from basophils stimulated with calcium ionophore A23187, zymosan activated human

serum and grass pollen allergens. Our results show a dose-dependent inhibition of histamine release, which is highly variable from subject to subject and from drug to drug.

Methods

Patients

Our study included eight normal subjects and six patients with grass pollen allergy (ages ranging from 23 to 35 years; mean $\pm$ s.e. mean 28 ± 1.1).

Preparation of leukocyte suspensions

Blood leukocytes, partially purified by dextran sedimentation, were suspended in Tyrode's buffer with 1.8 mM CaCl_2 and 0.5 mM MgCl_2 . Basophil granulocytes were counted in duplicate in a Fuchs–Rosenthal chamber, using the Alcian blue staining; a final concentration of 4×10^4 basophils ml^{-1} was obtained adjusting the volume of Tyrode's buffer.

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Histamine release from washed leukocytes

Basophil cells were stimulated with calcium ionophore A23187 (Sigma Chemical) at concentrations of $1 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, zymosan (Sigma Chemical) activated human serum (10% and 30%) and grass pollen allergens (Pharmacia) at concentrations of 10^4 and 10^3 bu ml^{-1} .

Histamine analysis was performed by an automated fluorometric method, modified from Siraganian & Brodsky (1976).

Study of the effect of in vitro pre-incubation with calcium antagonists on histamine release from washed basophil leukocytes

Partially purified basophil cells were incubated for 90 min at 37°C with different concentrations (from $1 \mu\text{M}$ to 1 mM) of verapamil (Knoll), methoxyverapamil (Knoll) and diltiazem (Lusofarmaco). Histamine release was subsequently induced as previously described. The effect of calcium antagonist drugs on basophil histamine release was also evaluated in presence of different extracellular calcium concentrations (from 0.9 mM to 5.4 mM).

Results

The *in vitro* pre-incubation of basophil cells with verapamil, methoxyverapamil and diltiazem, at dosages of 1, 10 and $100 \mu\text{M}$, exerted a concentra-

tion-dependent inhibition on the histamine release induced by calcium ionophore A23187, zymosan activated human serum and grass pollens. This inhibitory effect was highly variable from subject to subject and from drug to drug. No significant difference ($P > 0.05$) was found among the three calcium antagonists, concerning their protective activity.

After challenge with calcium ionophore ($10 \mu\text{g ml}^{-1}$) in 14 subjects, the percentages of inhibition (mean $\pm$ s.e. mean), at a drug concentration of $100 \mu\text{M}$, were 21.6 ± 6.4 for verapamil, 14.4 ± 2.7 for methoxyverapamil and 27.9 ± 4.7 for diltiazem (Figure 1a). With a lower dosage of calcium ionophore ($1 \mu\text{g ml}^{-1}$) the inhibitory activity was slightly higher for verapamil (26.2 ± 5.0) and methoxyverapamil (25.1 ± 4.1) and lower for diltiazem (22 ± 3.95).

The histamine release induced by zymosan activated serum (30%) in six subjects was reduced by calcium antagonists and the mean percentages of inhibition, at a drug concentration of $100 \mu\text{M}$, were 21.4 ± 6.5 for verapamil, 14.7 ± 5.1 for methoxyverapamil and 14.2 ± 5.8 for diltiazem (Figure 1b). The inhibitory activity of calcium antagonists was more remarkable when basophils were challenged with 10% zymosan activated serum, with values ranging between 15 and 42%. A protective effect was also observed on grass pollen (10^3 bu) induced histamine release in six allergic patients. At a drug concentration of $100 \mu\text{M}$, mean inhibition was $33.1 \pm 8.2\%$ for verapamil, $32.6 \pm 7.9\%$ for methoxy-

Figure 1 (a) Concentration-effect curves (mean $\pm$ s.e. mean) of verapamil (●—●), methoxyverapamil (●- - - ●) and diltiazem (● ··· ··· ●) on calcium ionophore A23187 ($10 \mu\text{g/ml}$)-induced histamine release from human basophil leukocytes. (b) Concentration-effect curves of verapamil (●—●), methoxyverapamil (●- - - ●) and diltiazem (● ··· ··· ●) on zymosan activated serum (30%)-induced histamine release from human basophil leukocytes.

verapamil and $27.4 \pm 8.9\%$ for diltiazem (Figure 2). With a lower dosage of grass pollens (10^4 bu), the reduction of histamine release was very similar to that observed with 10^3 bu.

The inhibitory effect of calcium entry blockers on histamine release was partially reversed by increasing calcium concentrations (data not shown).

We failed to calculate the IC_{50} , because high concentrations of calcium antagonists induced histamine release from basophils. The exposure of basophil leukocytes to high dosages ($> 200 \mu M$) of calcium entry blockers was followed by a concentration-dependent histamine release. Total histamine release was obtained with 0.5 mM verapamil and 2 mM methoxyverapamil and diltiazem.

We observed that calcium antagonists could induce histamine release even in the absence of extracellular calcium. The examination of the supernatants of the cells exposed to high dosages of calcium entry blockers revealed a concentration-dependent increase in LDH levels (from 10.2 ± 5.1 to 111 ± 12.5 u ml $^{-1}$).

Discussion

Our results indicate that verapamil, methoxyverapamil and diltiazem, at concentrations of 1–100 μM , can exert an inhibitory activity on IgE and non IgE mediated histamine release from human basophil granulocytes, in agreement with Jensen *et al.* (1983) and Chand *et al.* (1984).

The inhibitory effect is highly variable from subject to subject and from drug to drug. Individual variations in basophil releasability may account for the variability of results. In fact Findlay & Lichtenstein (1980) have observed large variations in basophil response, depending upon the stimulus and the subject. Moreover, pharmacokinetic characteristics, such as differences in lipid/water solubility (Pang & Sperelakis, 1984) and subsequent intracellular concentrations, could also be relevant factors in determining the variability of the inhibitory effect of the different calcium antagonists tested.

In our study the inhibitory effect of calcium antagonists was dependent on extracellular calcium concentration: thus it is reasonable to

Figure 2 (a) Concentration-effect curves (mean $\pm$ s.e. mean) of verapamil ($\bullet$ — $\bullet$), methoxyverapamil ($\bullet$ ---- $\bullet$) and diltiazem ($\bullet$ $\bullet$) on grass pollen (10000 BU)-induced histamine release from human basophil leukocytes.

suppose that these drugs exert their activity blocking transmembrane calcium flow.

In order to evaluate the IC_{50} , we exposed the basophil cells to high concentrations of calcium antagonists and we observed that these drugs could induce a dose-dependent histamine release, starting from concentrations of 200 μM . Also Lunardi & Vugman (1982) observed that verapamil can induce histamine release from rat mast-cells at dosages of 0.1–1 mM. Our experiments show that the calcium antagonist-induced histamine release is not dependent upon extracellular concentration of calcium ions and is associated with an increase in LDH activity in the cell supernatants. Therefore this histamine

releasing activity seems to be due to a cytolytic effect.

Other authors have described a similar toxic effect for high concentrations of ketotifen and clemastine (Moodley & Davies, 1982).

In conclusion, our *in vitro* results indicate that calcium antagonists have a complex effect on basophil degranulation: at concentrations ranging from 1 μM to 100 μM , they exert a variable inhibition on basophil histamine release, while at higher concentrations (> 200 μM) they induce histamine release. This effect is not dependent on extracellular concentration of calcium ions and seems to be associated with loss of cytoplasmic contents.

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