

Comparison of the action of calcium antagonists on basophil histamine release

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The inhibitory capacity of calcium antagonists on basophil histamine release was examined in allergic patients and in controls. All dihydropyridines tested (nifedipine, nimodipine, nitrendipine, nicardipine, felodipine) dose-dependently inhibited anti-IgE- and A23187-induced release with an order of potency of felodipine > nicardipine > nifedipine = nimodipine = nitrendipine. Only the inhibition induced by felodipine and nicardipine on anti-IgE-induced release could be counteracted by increasing extracellular calcium. Diltiazem, not belonging to the dihydropyridines, was a weak inhibitor. A combination of felodipine and verapamil in low concentrations exerted a synergistic inhibitory effect on histamine release, whereas this was not the case with other combinations of antagonists. The results suggest differences in the mode of action of the 1,4-dihydropyridines. This might be of significance in the search for calcium antagonists suitable in the treatment of allergic diseases.

Key words: basophil leukocytes; calcium antagonists; histamine release; inhibition.

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Histamine release from basophils and mast cells depends on the influx of calcium ions through specific calcium channels which are opened upon stimulation (3). We have previously shown that nifedipine, nimodipine, and verapamil inhibited basophil histamine release *in vitro* (6), and similar results have been obtained by others using various calcium channel antagonists in different cell systems (2, 8, 10, 17). The aim of this study was to investigate the inhibitory capacity and to make a comparison of most of the calcium antagonists currently available.

MATERIAL AND METHODS

Patients

This study included six normal individuals and six patients with a well-documented type 1

allergy to grass or birch pollen, or to animal dander verified by skin prick test. Basophils from all the patients released histamine when stimulated with the relevant allergen.

Materials

Nifedipine, nimodipine, and nitrendipine (Bayer AG, FRG), felodipine (Hässle AB, Sweden), and nicardipine (Syntex, Int., USA) were dissolved in ethanol (final concentration 0.1% at the experiments) and kept protected from the light. This concentration of ethanol did not affect histamine release induced by anti-IgE or A23187. Verapamil (Knoll AG, FRG) and diltiazem (Ferrosan A/S, Denmark) were dissolved in water. None of these compounds interfered with the assay. Rabbit anti-IgE (4000,000 IU/ml (ϵ -chain)) was from Behringwerke AG, FRG and the calcium

inophore A23187 was from Calbiochem AG, Switzerland.

Methods

Human leukocytes containing approx. 2% basophils were obtained by Ficoll-Hypaque gradient centrifugation (14). The cells were washed twice in Tris-AMC buffer (albumin, magnesium, calcium) and resuspended in this buffer (6, 14). Eighty μ l leukocyte suspension containing 2000–8000 basophils was incubated under red light in plastic tubes at 37°C for 5 min with 10 μ l TRIS-AMC or calcium antagonists. Histamine release was induced by adding 10 μ l of anti-IgE (a-IgE) or calcium ionophore A23187 to the samples and the incubation was continued for 40 min. If not otherwise stated, the final concentration of the stimulators constituted 40–400 IU/ml and 1 μ M, respectively. Calcium antagonists and stimulators were omitted for spontaneous histamine release and for determination of the total histamine content; in the latter case the samples were left at 4°C. Thereafter 3 ml ice-cold Tris-AMC was added to the samples and they were centrifuged (6, 14). Besides released histamine, the supernatant also contains protein and other substances which interfere with the histamine analysis. It was therefore discarded and the residual histamine was determined in the cell sediment according to the method of Stahl Skov & Norn (14). The release of histamine was expressed as a percentage of the total histamine content of the sample. The spontaneous histamine release amounted to 1–10%.

Statistics

Student's t-test was used. Mean $\pm$ SEM is given, if not otherwise stated.

RESULTS

The inhibitory capacity of calcium antagonists on histamine release in basophils from allergic patients and normal controls was examined *in vitro*. Histamine release was induced by either anti-IgE (40–400 IU/ml) or calcium ionophore

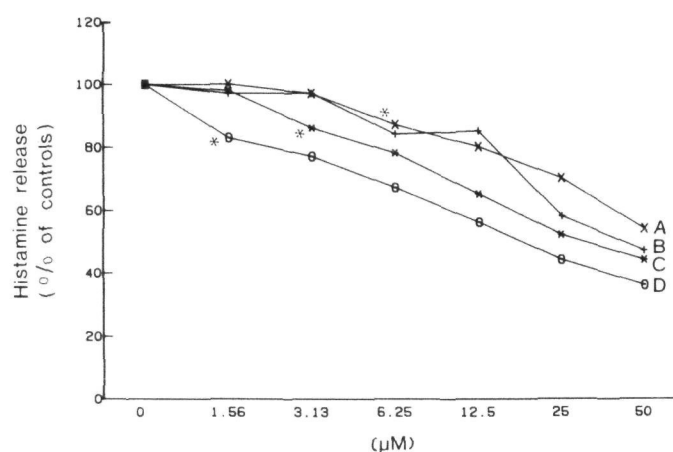


Fig. 1. Inhibition of anti-IgE-induced histamine release by nimodipine (A), nitrendipine (B), nicardipine (C), and felodipine (D). The curve of nifedipine, which did not differ from that of nimodipine, is not shown. Mean of 8 experiments (4 allergic patients and 4 controls). *Signify the lowest concentration of calcium antagonist giving a significant ($P < 0.05$) inhibition.

A23187 (1 μ M). In all experiments the release in the absence of calcium-antagonists varied between 30 and 70%. None of the calcium antagonists released histamine *per se*.

Fig. 1 demonstrates the inhibition of anti-IgE-induced release by the 1,4-dihydropyridines nimodipine, nitrendipine, nicardipine, and felodipine. At concentrations above 1.56 and 3.13 μ M, respectively, felodipine and nicardipine dose-dependently inhibited the release, whereas concentrations of nimodipine, nifedipine (data not shown) and nitrendipine exceeding 6.25 μ M were required to obtain significant inhibition. The maximal inhibition produced by the drugs was similar. There was very little interindividual difference in the inhibition profiles of the drugs, the standard error of the mean at all points never exceeding 9%, and also no difference was found in the inhibitory profile of the drugs between allergic patients and normal controls, and similar inhibition profiles were obtained when basophils from allergic patients were stimulated with specific allergen such as grass pollen or dog dander (data not shown).

The same pattern, although more pronounced, was seen in cells stimulated with A23187. Fig. 2 demonstrates that all the four drugs dose-dependently inhibited the release

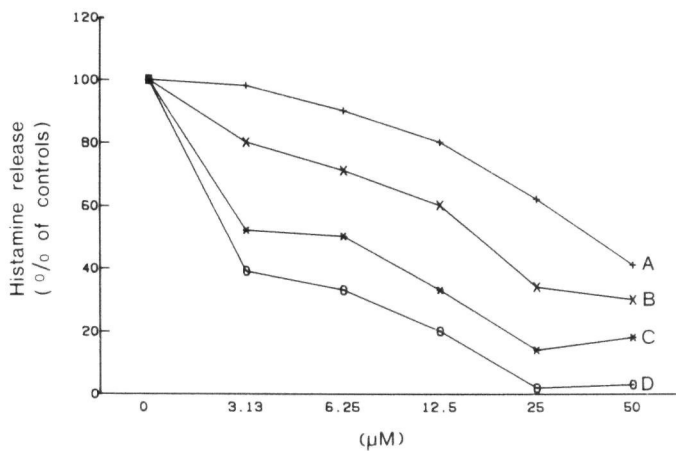


Fig. 2. Inhibition of A23187-induced histamine release by nimodipine (A), nitrendipine (B), nicardipine (C), and felodipine (D). Mean of 8 experiments (4 allergic patients and 4 controls). The curve of nifedipine, which did not differ from that of nimodipine, is not shown. In concentrations between 3.31 and 25 μM felodipine and nicardipine produced a significantly greater inhibition ($P < 0.02$) than nimodipine and nitrendipine.

and that felodipine and nicardipine had a higher potency than nimodipine and nitrendipine.

Fig. 3 shows the influence of increasing concentrations of extracellular calcium on the inhibitory effect of the 1,4-dihydropyridines. An increase from 0.6 to 1.8 mM reduced the inhibition induced by felodipine and nicardipine, and this reduction was even more pronounced at 6.0 mM of calcium, whereas the inhibition induced by nimodipine and nitrendipine (and nifedipine, data not shown) was unaffected even at 6.0 mM. In contrast, an increase in calcium to 6.0 mM abolished the inhibition of A23187-induced histamine release induced by all the five 1,4-dihydropyridines (data not shown). In both types of experiments there was no difference between allergic patients and controls.

The inhibitory capacity of diltiazem, a calcium channel antagonist not belonging to the 1,4-dihydropyridines, is shown in Fig. 4. It is seen that this compound is a weak inhibitor of basophil histamine release induced by anti-IgE (or A23187, data not shown). Only at a concentration of diltiazem of 0.1 mM was the release induced by anti-IgE or A23187 slightly inhibited, and this

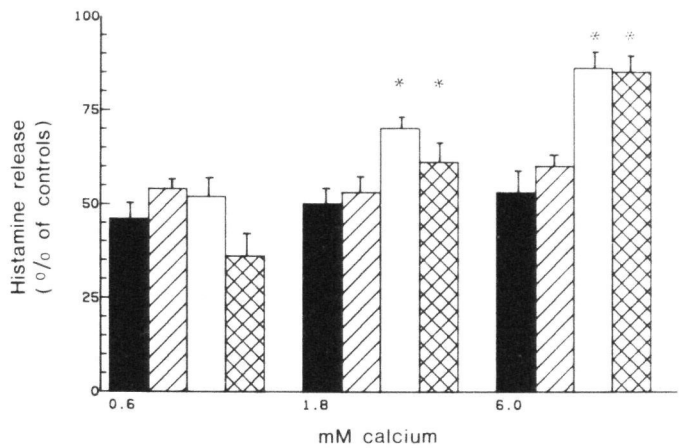


Fig. 3. Counteracting effect of increasing extracellular concentrations of calcium on the inhibition of anti-IgE-induced release by nicardipine ($\square$) and felodipine ($\square$), but not nimodipine ($\blacksquare$) or nitrendipine ($\square$). The curve of nifedipine, which did not differ from that of nimodipine, is not shown. Mean of 8 experiments (4 allergic patients and 4 controls) $\pm$ SEM. * $P < 0.01$ compared with 0.6 mM Ca^{2+} .

inhibition of the anti-IgE-induced release could be overcome by increasing the extracellular concentration of calcium to 6.0 mM.

The effect of a combination of calcium antagonist on anti-IgE-induced release is presented in Table 1. The individual drugs were used in concentrations causing only a very slight inhibition of the release. The concentration of verapamil was chosen from our results previously published (6). It can be seen from the table that a combination of nicardipine with either verapamil, diltiazem, or nimodipine only resulted in an additive effect. In contrast, felodipine combined with verapamil, but not with diltiazem or nimodipine, showed a synergistic effect, since felodipine (0.8 μM) and verapamil (10 μM) caused an inhibition of $9 \pm 1\%$ and $11 \pm 4\%$, respectively, whereas the combination of the two drugs resulted in an inhibition of $49 \pm 6\%$.

DISCUSSION

The activation of basophils and mast cells to release histamine is dependent on influx of extracellular calcium via the calcium channels (3). Several authors have investigated a possible inhibition of basophil histamine release by cal-

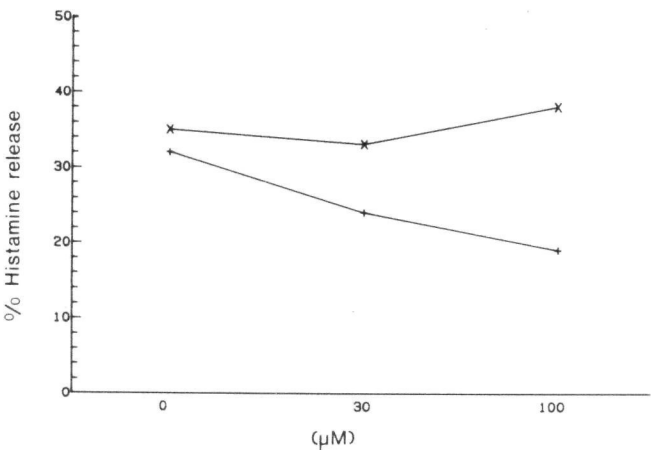


Fig. 4. Counteracting effect of increasing extracellular concentrations of calcium on the inhibitory effect of diltiazem. At 0.6 mM Ca²⁺ calcium (+) diltiazem inhibited anti-IgE-induced histamine release at a concentration of diltiazem of 100 μM, whereas this was not the case at 6.0 mM (x). A representative example of 5 experiments (3 allergic patients and 2 controls).

cium channel antagonists (for references see 10). Since, due to differences in experimental procedures, it is difficult to compare the results, most of the available calcium antagonists were in this study investigated under identical experimental conditions. The calcium antagonists have been classified in three groups according to their binding to three different receptor sites in the calcium channel (4). The groups are represented by verapamil, diltiazem and the 1.4-dihydropyridines (nifedipine, nimodipine, nitrendipine, nicardipine, and felodipine). In smooth muscle cells the existence of different types of calcium channels has been established

(15). One type of channel – the voltage-operated – is opened when the membrane potential is decreased, e.g. by increasing the extracellular concentration of potassium, whereas the so-called receptor-operated channel is activated by the binding of the ligand to its receptor (15). The currently available calcium channel antagonists are most effective on the voltage-operated channel, whereas the receptor-operated channel is much less sensitive (15). Thus, on rabbit aortic strips, concentration induced by stimulation of the voltage-operated channels is inhibited by the antagonist nifedipine in the nanomolar range, whereas those stimulated by the receptor-operated channels required micromolar concentrations (10).

All the 1.4-dihydropyridines investigated inhibited anti-IgE-induced basophil histamine release (Fig. 1). The potency of felodipine and nicardipine was significantly higher than that of nimodipine, nifedipine and nitrendipine, although the difference was not pronounced. The observed potencies are in accordance with those found by other authors (17) and with those inhibiting the influx of Ca-45 in red blood cells (16). Since micromolar concentrations of the compounds were necessary for inhibition, the presence of voltage-operated channels on the basophils does not seem likely, and this is supported by the finding that depolarization of the basophil membrane by 20 or 100 mM potassium did not induce histamine release (unpublished results) and by the lack of correlation between histamine release and membrane potential in rat mast cells and in rat basophil leukaemia cells (12, 13).

Therefore, a hypothesis might be that the observed effect of the calcium antagonists on the histamine release induced by anti-IgE might be due to inhibition of receptor-operated channels. It cannot be excluded that also other effects of the agents might contribute, since non-specific pharmacological effects, including blockade of Na⁺ and Cl⁻ channels, inhibition of calmodulin and of the oxidative phosphorylation, have been ascribed to different calcium antagonists (7). These non-specific effects might be of major importance in connection with inhibition of histamine release caused by the cal-

Table 1
Effect of a combination of two differently acting calcium antagonists on anti-IgE-induced histamine release

	Histamine release (% of controls)		
	0	Felodipine (0.8 μM)	Nicardipine (1.6 μM)
0	100	91 ± 1	90 ± 3
Verapamil (10 μM)	89 ± 4	51 ± 7*	84 ± 3
Diltiazem (10 μM)	93 ± 6	83 ± 8	88 ± 4
Nimodipine (3.12 μM)	90 ± 5	77 ± 4	73 ± 5

Mean of four experiments ± SEM. The combination of felodipine and verapamil showed a synergistic effect.

cium ionophore A23187, since this compound has been assumed to act by increasing intracellular calcium bypassing the channels (9). However, that this mode of action of the ionophore is its sole action has been questioned (11), and it might be possible that the calcium antagonists form chemical complexes with A23187, whereby this mechanism was produced.

In contrast to other 1,4-dihydropyridines, the inhibition of anti-IgE-induced histamine release by felodipine and nicardipine was counteracted by increasing the extracellular concentration of calcium (Fig. 3). These results with nicardipine are not in accordance with similar experiments on human mast cells (8), suggesting differences between the cell types. Our results suggest that modifications in the 1,4-dihydropyridine molecule might increase the affinity towards calcium channels not localized on smooth muscle cells.

The inhibitory capacity of diltiazem was less than that of the 1,4-dihydropyridines both with regard to potency and maximal efficacy (Fig. 4). The potency is comparable with that of verapamil (6), but it has a much lower maximal efficacy. Compared with rat mast cells, where diltiazem inhibited antigen-induced histamine release in concentrations of 10 μ M the human basophil is less sensitive (2). Since 1,4-dihydropyridines, verapamil and diltiazem act on different receptors in the calcium channel, it was investigated whether a combination of the compounds would increase the inhibition (Table 1). Since the action of felodipine and nicardipine seems to differ from that of nimodipine (Fig. 3), a combination of these compounds was also studied. Only the combination of felodipine and verapamil proved to be of interest, since a synergistic effect was obtained (Table 1), whereas this was not the case with the combination of nicardipine and verapamil. This indicates different modes of action of felodipine and nicardipine and suggests different subgroups of 1,4-dihydropyridines, which might be of significance in the search for calcium channel antagonists suitable in the treatment of allergic diseases.

In conclusion, the group of 1,4-dihydropyridines seem to be heterogeneous (Fig. 3 and

Table 1). The order of potency of the drugs tested seems to be felodipine > nicardipine > nimodipine = nifedipine = nitrendipine > verapamil > diltiazem. The effect of the calcium antagonists were similar in cells from allergic patients and normal controls. The apparent differences in the mode of action of the 1,4-dihydropyridines studied suggest that it might be of interest to investigate these compounds *in vivo* in allergic patients. Although nifedipine has only been of limited value in the prevention and treatment of asthma (1), felodipine or nicardipine – possibly in combination with verapamil – might prove useful.

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