

Inhibitory Effect of Calcium Antagonists on Histamine Release from Human Leukocytes

In Vivo and *in Vitro* Experiments

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A study was made of the influence of calcium antagonists on human basophil histamine release induced *in vitro* by specific antigen, anti-IgE or the calcium ionophore A23187. Both verapamil, nifedipine, and nimodipine were found to inhibit the release, and a similar effect was also observed after peroral administration of verapamil and nifedipine. The inhibitory effect of the drugs on histamine release seems to depend on interaction with calcium at different sites. The anti-allergic effect might explain the improvement found with calcium antagonists in exercise-induced asthma.

Key words: anti-allergic; antigen; anti-IgE; basophils; calcium antagonists; calcium ionophore; histamine release; *in vitro*; *in vivo*; mechanisms.

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Calcium antagonists are known to inhibit the influx of calcium ions across the cell membrane (12), and uptake of extracellular calcium in mast cells and basophil leukocytes is essential for the release of histamine and other mediators in the allergic type I reaction (8). It is therefore possible that these drugs might reduce the release of mediators in asthma and other atopic diseases. The present study examined the influence of verapamil, nifedipine, and nimodipine on human basophil histamine release induced by specific antigen, anti-IgE, and calcium ionophore. The study included *in vivo* as well as *in vitro* experiments, and the mode of action of the drugs, which seems to involve different mechanisms, was also studied.

MATERIAL AND METHODS

Patients

The investigation included six normal individuals and four patients with a well-docu-

mented history of symptoms of *Cladosporium herbarum* allergy. Using *Cladosporium herbarum* extract all subjects showed a positive response to RAST, bronchial provocation and skin prick tests.

Material

Nifedipine and nimodipine (Bayer AG, Germany) and verapamil (Knoll AG, Germany) *Cladosporium herbarum* extract (spectralgen *Cladosporium herbarum*, Pharmacia AB, Sweden), calcium ionophore A23187 (Calbiochem AG, Switzerland), rabbit-antihuman IgE (ϵ -chain) (Behringwerke AG, Germany).

Preparation of leukocyte suspension

By venipuncture, 10-ml blood samples were collected in plastic tubes containing 0.5 ml 0.20 M EDTA (pH 7.2). 20 ml of 0.9% NaCl was then added to the samples, and the leukocytes were isolated by the Ficoll-Hypaque gradient

method (2). 10 ml Ficoll-Hypaque solution (12.8 g Ficoll + 21 g Hypaque (sodium diatrizoate) dissolved in water to a final volume of approx. 200 ml to obtain a density of 1.080 g/ml at 20°C) was carefully placed below the blood mixture. The sample was centrifuged (400 *g* for 40 min) and the cotton wool-like interphase consisting of monocytes with approx. 2% basophils was mixed with 10 ml Tris-AMC (Tris (hydroxymethyl) aminomethane 25 mM at pH 7.6, NaCl 0.12 M, KCl 5 mM, CaCl₂ 0.6 mM, MgCl₂ 1.1 mM, human serum albumin 0.3 mg/ml, and glucose 1 mg/ml). The mixture was centrifuged (107 *g* for 10 min) and the cell sediment was resuspended in 10 ml Tris-AMC. The suspension was centrifuged (107 *g* for 10 min) and the supernatant containing thrombocytes was discarded. The leukocyte sediment was finally resuspended in 2.5 ml Tris-AMC.

Histamine release

80 µl leukocyte suspension containing 2000–8000 basophils was incubated in plastic tubes at 37°C for 5 min with 10 µl of Tris-AMC or calcium antagonists. Nifedipine and nimodipine were examined in final concentrations of 0.3 to 20×10^{-6} M and verapamil in 2.5×10^{-7} M to 2.5×10^{-4} M. Histamine release was induced by adding 10 µl of *Cladosporium herbarum*, 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 100 BU/ml, $10^{-3} \times 400,000$ IU/ml and 0.5×10^{-6} 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 at 2000 *g* for 10 min. 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 (18). To the cell sediment was added 400 µl of an OPT mixture

(2 mg *O*-phthaldialdehyde, Fluka, dissolved in 1 ml methanol and 9 ml 0.05 N NaOH).

After 4 min, 400 µl 0.175 N H₃PO₄ was added and the sample was centrifuged at 3000 *g* for 10 min. The histamine content in the supernatant was determined spectrofluorometrically in an Aminco-Fluoro-Colorimeter provided with a micro-flow cell. Samples were read at 355/455 nm (excitation/emission). 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%.

In vivo studies

In the *in vivo* studies blood was collected for estimation of histamine release before and after the treatment with calcium antagonists, i.e. at 09.00 h on the first and second day. Nifedipine, 20 mg $\times$ 4, or verapamil, 80 mg $\times$ 4, was given perorally within 24 h, i.e. at 09.30, 16.00, 23.00 and 07.00 h. *In vitro* release of histamine was estimated in the Ficoll-Hypaque isolated leukocytes after stimulation of the cells with anti-IgE or calcium ionophore, as mentioned above.

Statistical evaluation

Student's *t* test was employed. Mean $\pm$ SEM of duplicate determinations is given if not otherwise stated.

RESULTS

The release of histamine from basophil leukocytes was examined *in vitro* in both normal individuals and allergic patients. The release was induced by specific antigen in four *Cladosporium herbarum*-allergic patients and by anti-IgE or calcium ionophore A23187 in six normal individuals. In all experiments the maximal release varied between 25 and 80%. Fig. 1 shows typical release curves from one patient and one normal individual. The histamine release caused by these stimulators was inhibited in the presence of calcium antagonists. In all patients and normal individuals increasing con-

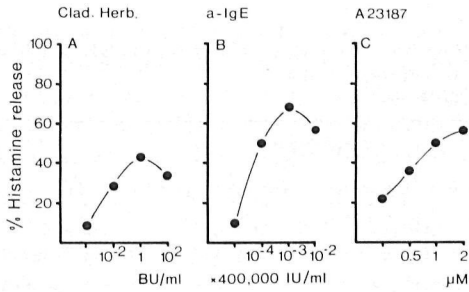


Fig. 1. Basophil histamine release caused by specific antigen (*Cladosporium herbarum*) in an allergic patient and by anti-IgE or calcium ionophore A23187 in a normal individual.

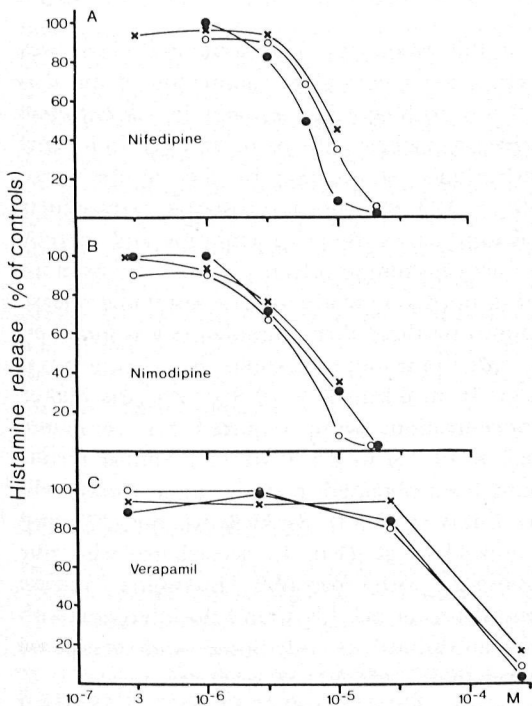


Fig. 2. Inhibition of histamine release by nifedipine, nimodipine, and verapamil. The release was caused by antigen (x—x) in four allergic patients, and by anti-IgE (●—●) and calcium ionophore (○—○) in six normal individuals.

centrations of calcium antagonists caused a nearly rectilinear reduction in histamine release in the semilogarithmic plot. For both nifedipine and nimodipine a 50% reduction was obtained at about 6×10^{-6} M, and in all experiments the release was completely abolished at 2×10^{-5} M (Fig. 2). Higher concentrations of verapamil were required where a complete inhibition was obtained at 2.5×10^{-4} M in all experiments (Fig. 2).

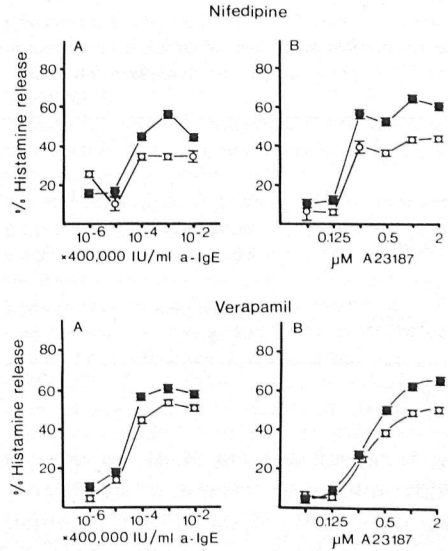


Fig. 3. Inhibition of histamine release after peroral administration of nifedipine (20 mg $\times$ 4) or verapamil (80 mg $\times$ 4) to a normal person. Before (●—●) and after (○—○) the drug administration. Each point represents the mean of four determinations $\pm$ SEM.

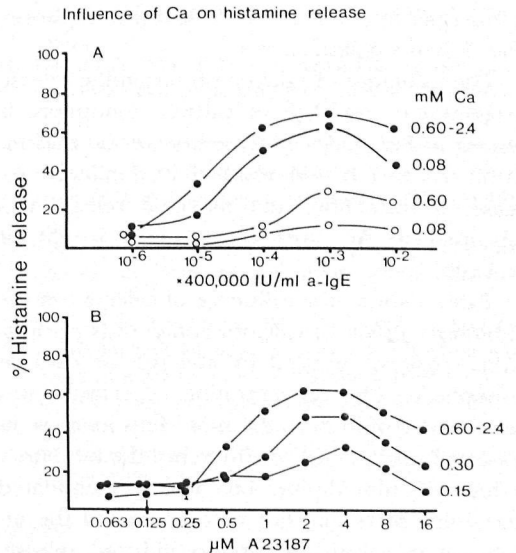


Fig. 4. Influence of calcium on histamine release induced by anti-IgE and by the calcium ionophore. A) two experiments (● and ○) and B) one experiment (●). Each point represents the mean of four determinations.

In the *in vivo* studies four normal individuals received peroral doses of nifedipine (20 mg $\times$

Table 1

Influence of calcium on the inhibitory effect of calcium antagonists on histamine release induced by anti-IgE or calcium ionophore. Mean of three experiments in duplicate $\pm$ SEM.
Inhibition (%) of histamine release

		Calcium (mM)			
		0.6	0.9	1.2	2.4
a-IgE	Nimodipine	61 $\pm$ 12	61 $\pm$ 14	65 $\pm$ 10	58 $\pm$ 12
	Verapamil	75 $\pm$ 3	75 $\pm$ 9	60 $\pm$ 2*	53 $\pm$ 1*
A23187	Nimodipine	52 $\pm$ 4	51 $\pm$ 15	16 $\pm$ 4*	19 $\pm$ 5*
	Verapamil	55 $\pm$ 4	54 $\pm$ 3	53 $\pm$ 1	26 $\pm$ 7*

* $P < 0.01$.

4) or verapamil (80 mg $\times$ 4). In these four persons nifedipine caused a significant decrease ($P < 0.01$) in the release of histamine when the basophil leukocytes were challenged *in vitro* with anti-IgE or calcium ionophore; the inhibition amounted to $35 \pm 6\%$ and $19 \pm 3\%$, respectively. Representative curves are shown in Fig. 3. A significant decrease ($P < 0.01$) was obtained with verapamil in two of the four individuals, where the inhibition was $23 \pm 8\%$ (anti-IgE) and $28 \pm 7\%$ (calcium ionophore). Fig. 3 shows typical curves.

The influence of calcium on histamine release triggered by anti-IgE or calcium ionophore is shown in Fig. 4. An increase in external calcium from 0.1 to 0.6 mM resulted in enhanced release of histamine, and maximal release was obtained in the area of 0.6 to 2.4 mM of calcium.

Table 1 shows the influence of calcium on the inhibitory effect of calcium antagonists on histamine release induced by anti-IgE or calcium ionophore. The concentration of calcium was increased from 0.6 to 2.4 mM. This increase in external calcium did not influence the inhibitory effect of nimodipine on anti-IgE-stimulated histamine secretion, but it counteracted the inhibition in calcium ionophore-induced release. The inhibitory effect of verapamil was in both instances reduced by the increase in calcium.

DISCUSSION

This study examined the influence of calcium antagonists on histamine release from human

basophil leukocytes. The histamine release was examined *in vitro* after stimulation of the leukocytes with specific antigen in *Cladosporium herbarum*-allergic patients or anti-IgE in normal individuals. Stimulation by the calcium ionophore A23187, which transports extracellular calcium across the cell membrane and thereby induces histamine release (8), was also examined in normal individuals. The histamine release caused by these three stimulators was inhibited by nifedipine and nimodipine in low concentrations from 0.3 to 2×10^{-5} M, whereas higher concentrations were required for verapamil (2.5×10^{-5} M to 2.5×10^{-4} M). Similar results have been obtained in rat peritoneal mast cells by Ennis et al. (3), Saeki & Kurose (17), and Tanizaki et al. (19). In accordance with our findings with basophil histamine release Middleton et al. (11) found no inhibition with concentrations of nifedipine and verapamil lower than 1 and 50 μ M, respectively.

In the *in vivo* studies blood was collected before and after peroral administration of nifedipine or verapamil, and the *in vitro* release of histamine was then estimated after stimulation of the basophil leukocytes with anti-IgE or calcium ionophore. A significant inhibition of the release was obtained in the four persons receiving nifedipine and in two of the four individuals receiving verapamil.

The results indicate that calcium antagonists are able to inhibit the release of histamine. These drugs might therefore have a beneficial effect in asthma and other atopic diseases, and recent reports already indicate an improvement

in exercise-induced asthma (1, 13, 14, 15).

To investigate the mechanism of the inhibitory effect of the drugs on histamine release we used different types of stimulators. Antigen and anti-IgE trigger the mediator release by opening receptor-operated calcium channels, whereas the calcium ionophore is thought to act as a calcium carrier, which bypasses these channels (4, 6, 7, 8, 9). Since the release induced by these three types of stimulators was inhibited by the drugs, we suggest that they act at different sites and/or at a common subsequent, and possibly intracellular step. The inhibition was reduced by increasing concentrations of external calcium, except for nimodipine, in the anti-IgE-mediated histamine release process. Therefore, nimodipine might not act as a simple calcium channel antagonist in the secretion of histamine, whereas such a mechanism cannot be excluded for verapamil. New investigations also suggest that the mode of action for the calcium antagonists is more complex than previously assumed and that it might depend on interaction at multiple sites (5, 10, 16). These drugs might be a new approach to the treatment of allergic diseases.

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