Thorax 1983;**38**:386–387

Short reports

Verapamil inhibits mediator release from human lung in vitro

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Calcium depletion inhibits the antigen-induced release of histamine and slow-reacting substance of anaphylaxis (SRS-A) from human lung tissue in vitro, indicating the critical role of transmembrane calcium ion influx in mediator release.[1] Since experimental data have implicated histamine and SRS-A (now identified as a mixture of leukotrienes C_4, D_4, and E_4)[2] in the pathogenesis of asthma,[3] the therapeutic potential of calcium antagonists in clinical models of asthma such as exercise-induced asthma has been investigated recently.[4–7] In a study of 10 asthmatic patients, it was shown that inhalation of the calcium antagonist verapamil afforded significant protection against exercise-induced asthma without causing bronchodilation.[4] On the basis of these observations two possible mechanisms for this protective effect were suggested: firstly, verapamil may have suppressed mediator release within the lung; or, secondly, verapamil may have inhibited the contractile effects of mediators such as histamine and leukotrienes on bronchial smooth muscle.[4] This latter possibility seems unlikely since pretreatment with inhalation of verapamil failed to modify histamine-induced or methacholine-induced bronchoconstriction in asthmatic patients.[7] The present experiments were undertaken to investigate the former possibility by determining whether verapamil could inhibit the release of histamine and SRS-A from human lung tissue in vitro.

Methods and results

Macroscopically normal lung tissue, obtained from operative specimens resected for carcinoma of the lung, was cut into small pieces and passively sensitised by overnight incubation in human serum with a high titre of immunoglobulin E specific for *Dermatophagoides pteronyssinus*, as described previously.[8] The following day the lung fragments were washed and then preincubated at 37°C in Tyrode solution containing different concentrations of verapamil for 15 minutes before the addition of *D pteronyssinus* extract (200 Noon units/ml). After a further 15 minutes at 37°C the supernatant was removed and the content of histamine and SRS-A was determined by fluorimetric and bioassay techniques respectively, as described previously.[8] The bioassay comprised strips of guinea-pig ileal longitudinal smooth muscle, superfused with mepyramine 1 μmol/l in Tyrode solution to antagonise the effects of histamine. SRS-A activity was calibrated with synthetic leukotriene E_4 (LTE_4) and expressed as ng equivalents of LTE_4. There were duplicate tubes for each concentration of verapamil. The study was approved by the medical ethical review committee of the University of Sydney. The significance of differences between control values and those obtained in the presence of verapamil was determined with Student's *t* test.

In lung fragments from five operative specimens antigen challenge released 25·2 ± 4·6% (mean ± SEM) of original tissue histamine. The release of SRS-A was 197·2 ± 50·9 ng (mean ± SEM) equivalents of LTE_4. Verapamil in concentrations of 10 and 100 μmol/l caused a statistically significant dose-related reduction in SRS-A release (table). At the highest concentration (100 μmol/l) verapamil reduced the release of histamine. No concentration of verapamil interfered with the fluorimetric assay for histamine. In concentrations of 10 μmol/l verapamil did not antagonise the action of leukotrienes on the strips of guinea-pig ileum. In some experiments 100 μmol/l caused some reduction in the contractile activity of leukotrienes (mean reduction 31%). In these experiments this direct effect on the bioassay tissues was taken into account in the calculation of the final value for the inhibitory effect of verapamil 100 μmol/l on the release of SRS-A from lung tissue.

Discussion

These studies have shown that verapamil caused a concentration-dependent inhibition of SRS-A release from human lung tissue in vitro. Whereas a lower concentration of verapamil (10 μmol/l) was effective in significantly reducing SRS-A release, a higher concentration (100

Effect of verapamil on mediator release from five lung specimens (values are means ± SEM, expressed as percentage of antigen-induced release in the absence of verapamil)

	Verapamil (μmol/l)		
	1	10	100
SRS-A	88·9 ± 4·9	73·0 ± 3·9*	23·1 ± 7·3†
Histamine	108·7 ± 3·9	103·4 ± 3·2	60·2 ± 5·4*

*$p < 0.005$; †$p < 0.001$.

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Accepted 1 October 1982

Thorax: first published as 10.1136/thx.38.5.386 on 1 May 1983. Downloaded from http://thorax.bmj.com/ on April 10, 2024 by guest. Protected by copyright.

μmol/l) was required for significant inhibition of histamine release. By direct measurement of mediator release from lung tissue in vitro the experiments with verapamil support the findings of a previous study of asthmatic patients in which another calcium antagonist, nifedipine, reduced the severity of exercise-induced bronchoconstriction and prevented the rise in venous plasma histamine concentration which accompanied exercise-induced asthma.[6] Rises in plasma histamine during exercise in asthmatics may, however, reflect release from circulating basophils rather than pulmonary mast cells, in which case nifedipine may have been inhibiting basophils rather than mast cells within the lung.[6] The results of the present experiments have indicated that verapamil has a direct inhibitory effect on the release of leukotrienes and histamine from mast cells in human lung tissue.

Despite these similar effects on mediator release, verapamil and nifedipine differ in their actions on bronchial smooth muscle. Whereas inhalation of verapamil did not alter the histamine-induced bronchoconstriction in one study of asthmatic patients,[7] sublingual nifedipine afforded a small but significant protective effect against histamine challenge in another study.[6] Apparently there are qualitative differences between drugs classified loosely as calcium antagonists.

In contrast to histamine, which is preformed and stored within the granules of mast cells, SRS-A is synthesised de novo at the time of antigen stimulation. The leukotrienes, which have been identified as the active moieties in SRS-A, are derived from arachidonic acid.[2] Before conversion to leukotrienes arachidonic acid is liberated from phospholipids by phospholipase A_2, a calcium-dependent enzyme.[9] Possibly therefore verapamil suppressed phospholipase A_2 activity by inhibiting the influx of calcium ions. Furthermore, the present data suggest that the calcium channels concerned in the biosynthesis and release of SRS-A are more susceptible to inhibition by calcium antagonists than are the calcium channels regulating histamine release.

Whether this action of verapamil contributes to its protective effect in exercise-induced asthma remains speculative. When verapamil is given orally or intravenously in clinically recommended doses, the peak plasma concentrations range from 80 to 160 nmol/l.[10] Possibly, however, higher concentrations of active drug are achieved in target cells within the lung after inhalation of nebulised verapamil. Moreover, these findings with verapamil suggest that calcium antagonists may be useful in clinical disorders in which the release of leukotrienes and histamine is thought to be concerned.

We thank the Asthma Foundation of New South Wales for financial support, Knoll AG for the verapamil, Dr BA Baldo for the synthetic leukotriene E_4, and Dr JL Black for assistance in obtaining human lung.

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