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Enzyme-Potentiated Hyposensitisation

I. The Effect of Pre-treatment with β-Glucuronidase, Hyaluronidase, and Antigen on Anaphylactic Sensitivity of Guinea-Pigs, Rats and Mice

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Abstract. The ability of preparations of hyaluronidase and β-glucuronidase to potentiate the hyposensitising effect of small doses of antigen injected subcutaneously into previously sensitised animals has been investigated in three different models of experimental anaphylaxis.

Guinea-pigs were sensitised by egg albumen and repeatedly challenged by exposure to an aerosol of the antigen at weekly intervals. A small dose of antigen by injection increased their pre-convulsion times on subsequent challenge, and this was potentiated by the addition of hyaluronidase and β-glucuronidase.

Rats were sensitised by horse serum and challenged 18 days later with intravenous antigen, deaths being noted within 24 h. Ten days after sensitisation, subcutaneous injections of antigen and β-glucuronidase greatly reduced the mortality on subsequent challenge although antigen injected alone had no effect.

In mice sensitised by horse serum and challenged by pinnal anaphylaxis, the anamnestic increase in sensitivity produced by a subcutaneous dose of antigen was prevented by β-glucuronidase. Using this model, tolerance was induced by the intravenous injection of antigen and this was prevented by the addition of hyaluronidase.

Introduction

In an earlier study [McEwen *et al.*, 1967], it was reported that patients who had specific allergic diseases of the immediate type were considerably improved by the application of a mixture of hyaluronidase and specific antigen to a scarification site on the forearm. The sample of hyaluronidase used in this study was found to be contaminated with a variable mixture of enzymes, and one of these, β-glucuronidase, was shown to confer the beneficial therapeutic activity. In fact, pure

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hyaluronidase was found to be inactive in promoting clinical hyposensitisation [unpublished observation].

The purpose of the present work has been to study animal models in which this type of hyposensitisation may be identified and to determine the effect of hyaluronidase and β-glucuronidase on the hyposensitising ability of the antigen.

Materials and Methods

Horse serum without preservative (Burroughs Wellcome Ltd.); egg albumen (British Drug Houses Ltd.); hyaluronidase (Fisons Pharmaceuticals Ltd.); β-glucuronidase from *Patella vulgata* 2,000 Fishman U/mg (Koch Light Laboratories Ltd.); pontamine sky blue dye, type 6BX (George T. Gurr Ltd.). The dye was dissolved in a small volume of saline and dialysed against normal saline for 48 h. Then it was cleared of large particles and sterilised by passage through a Millipore filter (type HA) with pore size of 0.45 μm. It was stored sterile and ready for use at a concentration of 25 mg/ml in saline.

The following buffer was used for solutions of β-glucuronidase: NaCl 2.0 g; KCl 4.0 g; sodium acetate 0.2 g; $MgSO_4$ 0.06 g; NaH_2PO_4 0.3 g; $CaCl_2$ 0.18 g; distilled water to 1 l.

Treatment of Animals

Male albino guinea-pigs weighing 250–400 g were sensitised subcutaneously by egg albumen (100 mg). After 3 weeks, they were challenged at weekly intervals by exposure to a 1.0-percent aqueous aerosol of the antigen [HERXHEIMER, 1952]. Animals showing discomfort were quickly removed from the aerosol atmosphere and revived with oxygen. The pre-convulsion times were recorded, and animals failing to sensitise adequately (pre-convulsion times of more than 3 min) were discarded. Consistent values for pre-convulsion times for each animal were obtained after 3–5 consecutive exposures. Then the animals received a desensitising dose of antigen (0.1 mg) subcutaneously, with or without the addition of hyaluronidase (1,500 U) or β-glucuronidase (500 U). Challenge was begun again after 24 h and continued weekly for at least 8 weeks, the changes in pre-convulsion times being recorded.

Male Wistar albino rats weighing 150–250 g were sensitised by horse serum (0.5 ml) intraperitoneally and with the aid of an adjuvant (*Bordetella pertussis* vaccine, 0.25 ml containing 10^6 organisms). Ten days later, the animals were injected with saline or horse serum (0.1 ml) subcutaneously with or without the addition of hyaluronidase (1,500 U) or β-glucuronidase (500 U). Challenge was made intravenously 8 days later with horse serum (1 ml). The number of deaths in each group over the next 2 and 24 h were recorded.

Male Porton mice weighing 18–20 g were sensitised by horse serum (250 μg protein) subcutaneously. A second dose of antigen (1 μg subcutaneously or 100 μg intravenously) with or without hyaluronidase (5 U) or β-glucuronidase (10 U) was administered 3 weeks later. After a further 8 days, the mice were tak-

en to a warm room (30 °C) and injected intravenously with pontamine sky blue 100 mg/kg. They were challenged cutaneously 1 h later by piercing through each pinna a drop of undiluted horse serum [Feinberg, 1961]. 45 min later, the mice were sacrificed and their pinnae were removed and mounted on cards. The blued areas were examined by an independent observer. Under a bright light, the edge of each blue patch was circumscribed in ink and the area determined using the best fit of standard circles.

Results

Guinea-Pigs

Most sensitised animals convulsed within 90 sec of being exposed to the antigen aerosol and maintained this degree of sensitivity for at least 12 weeks. Animals of differing sensitivity were distributed randomly among the experimental groups. The subcutaneous injection of antigen always resulted in a slight prolongation of the mean pre-convulsion times over the next 3–4 weeks, after which resensitisation generally occurred (fig. 1, graph 1). When β-glucuronidase was included in the desensitising injection dose, the degree of hyposensitisation was greater and of longer duration than with antigen alone (graph 2), and the pre-convulsion times did not return to control levels for 5–6 weeks. When hyaluronidase was also included in the injected dose, an even more rapid and extensive decrease in sensitivity to the antigen aerosol was obtained (graph 3), and this change in sensitivity lasted for 6–8 weeks.

Rats

When rats were treated subcutaneously with saline or specific antigen 10 days after sensitisation and challenged intravenously with antigen 8

Table I. Effect on the mortality rate of rats of subcutaneous injections of either saline or specific antigen, with or without β-glucuronidase or hyaluronidase 10 days after sensitisation. Measured 2 and 24 h after intravenous challenge with antigen 18 days after sensitisation

Treatment	Number of rats	Mortality rate, %	
		2 h	24 h
Saline	10	40	70
Horse serum	10	50	70
Horse serum + hyaluronidase	10	30	40
Horse serum + β-glucuronidase	6	0	16

Table II. Effect on the challenge by pinnal anaphylaxis in groups of 13 mice 8 days after subcutaneous injections of either saline or specific antigen, with or without β-glucuronidase, 3 weeks after sensitisation

Treatment	Mean area of blueing, mm^2
Saline	45
Horse serum 1 μg	74[1]
Horse serum 1 μg +β-glucuronidase	49[1]

[1] Test for significance $t = 7.2$; $p = > 0.001$.

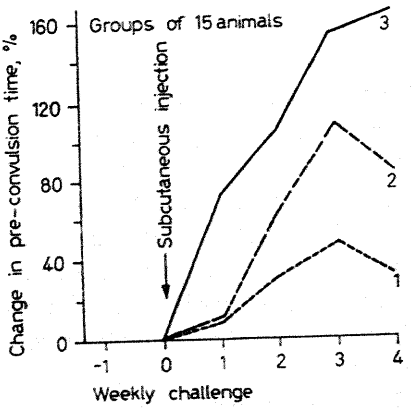

Fig. 1. Effect of subcutaneous injections of specific antigen, β-glucuronidase and hyaluronidase on the pre-convulsion times of sensitised guinea-pigs, challenged by aerosol of antigen at weekly intervals. Graph 1 is after antigen (egg albumen 0.1 mg), graph 2 after antigen (egg albumen) and β-glucuronidase (500 U), and graph 3 after antigen (egg albumen), β-glucuronidase (500 U) and hyaluronidase (1,500 U).

days later, 70% of the animals died within the next 24 h (table I). Treatment with horse serum and hyaluronidase afforded some protection, but when β-glucuronidase was used with the horse serum for treatment, subsequent challenge resulted in no deaths at 2 h and only one animal out of a group of 6 died within 24 h.

Mice

A second dose of horse serum (1 μg protein) by subcutaneous injection provoked in mice an anamnestic increase in anaphylactic sensitivity, and this response was prevented by the inclusion of β-glucuronidase with the antigen (table II). Table III shows the effect of administering a second dose of horse serum (100 μg protein) intravenously. With this route of administration, the antigen by itself caused a striking degree of desensitisation. The addition of β-glucuronidase (10 U) to the intravenous dose induced a slight increase in desensitisation, but when hyaluronidase (5 U) was added to the intravenous treatment, the effect of the antigen was reversed, and an anamnestic rise in anaphylactic sensitivity resulted.

Discussion

The results of the present study indicate that the enzymes tested are capable of enhancing the degree of hyposensitisation induced when small sublethal quantities of specific antigen are injected into sensitised guinea-pigs and rats. Although the same has been found to be true for mice when very small quantities of antigen are used [unpublished observations], adsorption of antigen protein results in inconsistancy, and in the present work a larger quantity (1 μg protein) has been used subcutaneously. This induces an anamnestic rise in anaphylactic sensitivity which is prevented by the simultaneous administration of β-glucuronidase.

Mitchison [1968] suggested that tolerance induction may occur over a wide range of antigen doses, but above a certain threshold this effect is merely masked by the immune response. The ability of β-glucuronidase to prevent the response to a dose of antigen which is high enough to provoke antibody production indicates that at lower dose levels of antigen the induction of tolerance may be enhanced indirectly by the suppression of simultaneous weak immunity and not by a direct effect.

The present results give experimental support to the suggestion of McEwen *et al.* [1967] that the ability of commercial samples of hyaluronidase to potentiate clinical hyposensitisation depends on their contamination with different quantities of β-glucuronidase.

The guinea-pig results show that, although β-glucuronidase (in the absence of hyaluronidase) is able to potentiate hyposensitisation due to antigea, the effect is improved when hyaluronidase is also present.

Table III. Effect on the challenge by pinnal anaphylaxis in groups of 5 mice 8 days after intravenous injections of either saline or specific antigen, with or without β-glucuronidase and hyaluronidase, 3 weeks after sensitisation

Group	Treatment	Mean area of blueing, mm^2
1	Saline	34
2	Horse serum 100 μg	16
3	Horse serum 100 μg +β-glucuronidase	11
4	Horse serum 100 μg +β-glucuronidase +hyaluronidase	43

Tests for significance: 1 vs 2 $p = >0.05$; 1 vs. 3 $p = >0.001$; 2 vs. 4 $p = >0.01$; 3 vs. 4 $p = >0.01$.

In the rat, commercial hyaluronidase is less effective than β-glucuronidase in conferring hyposensitising activity on a dose of antigen which by itself had no protective effect.

Intravenous injections with antigen and enzyme mixtures in mice show that in certain circumstances hyaluronidase is deleterious to the induction of immunological tolerance, whereas β-glucuronidase is not. The result suggests that tolerance is only induced by this method when the injected antigen remains trapped in the vascular compartments of the body. Increased vascular permeability due to the hyaluronidase then allows the antigen to escape and immunity results. The effect of other, less complicated, substances which increase vascular permeability on the induction of tolerance by intravenous injection of antigen might repay further study.

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